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(54) Title: NOVEL DNA CLONING METHOD

(57) Abstract

The invention refers to a novel method for cloning DNA molecules using a homologous recombination mechanism between at least two DNA molecules comprising: a) providing a host cell capable of performing homologous recombination, b) contacting in said host cell a first DNA molecule which is capable of being replicated in said host cell with a second DNA molecule comprising at least two regions of sequence homology to regions on the first DNA molecule, under conditions which favour homologous recombination between said first and second DNA molecules and c) selecting a host cell in which homologous recombination between said first and second DNA molecules has occurred.

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In the method of the present invention the homologous recombination preferably occurs via the recET mechanism, i.e. the homologous recombination is mediated by the gene products of the recE and the recT genes which are preferably selected from the E.coli genes recE and recT or functionally related genes such as the phage λ redα and redß genes.

The host cell suitable for the method of the present invention preferably is a bacterial cell, e.g. a gram-negative bacterial cell. More preferably, the host cell is an enterobacterial cell, such as Salmonella, Klebsiella or Escherichia. Most preferably the host cell is an Escherichia coli cell. It should be noted, however, that the cloning method of the present invention is also suitable for eukaryotic cells, such as fungi, plant or animal cells.

Preferably, the host cell used for homologous recombination and propagation of the cloned DNA can be any cell, e.g. a bacterial strain in which the products of the recE and recT, or redα and redß, genes are expressed. The host cell may comprise the recE and recT genes located on the host cell chromosome or on non-chromosomal DNA, preferably on a vector, e.g. a plasmid. In a preferred case, the RecE and RecT, or Redα and Redß, gene products are expressed from two different regulatable promoters, such as the arabinose-inducible BAD promoter or the lac promoter or from non-regulatable promoters. Alternatively, the recE and recT, or redα and redß, genes are expressed on a polycistronic mRNA from a single regulatable or non-regulatable promoter. Preferably the expression is controlled by regulatable promoters.

Especially preferred is also an embodiment, wherein the recE or redα gene is expressed by a regulatable promoter. Thus, the recombinogenic potential of the system is only elicited when required and, at other times, possible undesired recombination reactions are limited. The recT or redß gene, on the other hand, is preferably overexpressed with respect to recE or redα. This may be accomplished by using a strong constitutive promoter, e.g. the

EM7 promoter and/or by using a higher copy number of recT, or redß, versus recE, or redα, genes.

For the purpose of the present invention any recE and recT genes are suitable insofar as they allow a homologous recombination of first and second DNA molecules with sufficient efficiency to give rise to recombination products in more than 1 in 10^9 cells transfected with DNA. The recE and recT genes may be derived from any bacterial strain or from bacteriophages or may be mutants and variants thereof. Preferred are recE and recT genes which are derived from E.coli or from E.coli bacteriophages, such as the redα and redß genes from lambdoid phages, e.g. bacteriophage λ.

More preferably, the recE or redα gene is selected from a nucleic acid molecule comprising

(a) the nucleic acid sequence from position 1320 (ATG) to 2159 (GAC) as depicted in Fig.7B,

(b) the nucleic acid sequence from position 1320 (ATG) to 1998(CGA) as depicted in Fig.14B,

(c) a nucleic acid encoding the same polypeptide within the degeneracy of the genetic code and/or

(d) a nucleic acid sequence which hybridizes under stringent conditions with the nucleic acid sequence from (a), (b) and/or (c).

More preferably, the recT or redß gene is selected from a nucleic acid molecule comprising

(a) the nucleic acid sequence from position 2155 (ATG) to 2961 (GAA) as depicted in Fig.7B,

(b) the nucleic acid sequence from position 2086 (ATG) to 2868 (GCA) as depicted in Fig.14B,

(c) a nucleic acid encoding the same polypeptide within the degeneracy of the genetic code and/or

(d) a nucleic acid sequence which hybridizes under stringent conditions with the nucleic acid sequences from (a), (b) and/or (c).

It should be noted that the present invention also encompasses mutants and variants of the given sequences, e.g. naturally occurring mutants and variants or mutants and variants obtained by genetic engineering. Further it should be noted that the recE gene depicted in Fig.7B is an already truncated gene encoding amino acids 588-866 of the native protein. Mutants and variants preferably have a nucleotide sequence identity of at least 60%, preferably of at least 70% and more preferably of at least 80% of the recE and recT sequences depicted in Fig.7B and 13B, and of the redα and redß sequences depicted in Fig.14B.

According to the present invention hybridization under stringent conditions preferably is defined according to Sambrook et al. (1989), infra, and comprises a detectable hybridization signal after washing for 30 min in 0.1 x SSC, 0.5% SDS at 55°C, preferably at 62°C and more preferably at 68°C.

In a preferred case the recE and recT genes are derived from the corresponding endogenous genes present in the E.coli K12 strain and its derivatives or from bacteriophages. In particular, strains that carry the sbcA mutation are suitable. Examples of such strains are JC8679 and JC 9604 (Gillen et al. (1981), supra). Alternatively, the corresponding genes may also be obtained from other coliphages such as lambdoid phages or phage P22.

The genotype of JC 8679 and JC 9604 is Sex (Hfr, F+, F-, or F') : F-.JC 8679 comprises the mutations: recBC 21, recC 22, sbcA 23, thr-1, ara-14, leu B 6, DE (gpt-proA) 62, lacY1, tsx-33, gluV44 (AS), galK2 (Oc), LAM-, his-60, relA 1, rps L31 (strR), xyl A5, mtl-1, argE3 (Oc) and thi-1. JC 9604 comprises the same mutations and further the mutation recA 56.

Further, it should be noted that the recE and recT, or redα and redß, genes can be isolated from a first donor source, e.g. a donor bacterial cell and transformed into a second receptor source, e.g. a receptor bacterial or eukaryotic cell in which they are expressed by recombinant DNA means.

In one embodiment of the invention, the host cell used is a bacterial strain having an sbcA mutation, e.g. one of E.coli strains JC 8679 and JC 9604 mentioned above. However, the method of the invention is not limited to host cells having an sbcA mutation or analogous cells. Surprisingly, it has been found that the cloning method of the invention also works in cells without sbcA mutation, whether recBC+ or recBC-, e.g. also in prokaryotic recBC+ host cells, e.g. in E.coli recBC+ cells. In that case preferably those host cells are used in which the product of a recBC type exonuclease inhibitor gene is expressed. Preferably, the exonuclease inhibitor is capable of inhibiting the host recBC system or an equivalent thereof. A suitable example of such exonuclease inhibitor gene is the λ redγ gene (Murphy, J.Bacteriol. 173 (1991), 5808-5821) and functional equivalents thereof, respectively, which, for example, can be obtained from other coliphages such as from phage P22 (Murphy, J.Biol.Chem.269 (1994), 22507-22516).

More preferably, the exonuclease inhibitor gene is selected from a nucleic acid molecule comprising

(a) the nucleic acid sequence from position 3588 (ATG) to 4002 (GTA) as depicted in Fig.14A,

(b) a nucleic acid encoding the same polypeptide within the degeneracy of the genetic code and/or

(c) a nucleic acid sequence which hybridizes under stringent conditions (as defined above) with the nucleic acid sequence from (a) and/ or (b).

Surprisingly, it has been found that the expression of an exonuclease inhibitor gene in both recBC+ and recBC- strains leads to significant improvement of cloning efficiency.

The cloning method according to the present invention employs a homologous recombination between a first DNA molecule and a second DNA molecule. The first DNA molecule can be any DNA molecule that carries an origin of replication which is operative in the host cell, e.g. an E.coli replication origin. Further, the first DNA molecule is present in a form which is capable of being replicated in the host cell. The first DNA molecule, i.e. the vector, can be any extrachromosomal DNA molecule containing an origin of replication which is operative in said host cell, e.g. a plasmid including single, low, medium or high copy plasmids or other extrachromosomal circular DNA molecules based on cosmid, P1, BAC or PAC vector technology. Examples of such vectors are described, for example, by Sambrook et al. (Molecular Cloning, Laboratory Manual, 2nd Edition (1989), Cold Spring Harbor Laboratory Press) and Ioannou et al. (Nature Genet. 6 (1994), 84-89) or references cited therein. The first DNA molecule can also be a host cell chromosome, particularly the E.coli chromosome. Preferably, the first DNA molecule is a double-stranded DNA molecule.

The second DNA molecule is preferably a linear DNA molecule and comprises at least two regions of sequence homology, preferably of sequence identity to regions on the first DNA molecule. These homology or identity regions are preferably at least 15 nucleotides each, more preferably at least 20 nucleotides and, most preferably, at least 30 nucleotides each. Especially good results were obtained when using sequence homology regions having a length of about 40 or more nucleotides, e.g. 60 or more nucleotides. The two sequence homology regions can be located on the linear DNA fragment so that one is at one end and the other is at the other end, however they may also be located internally. Preferably, also the second DNA molecule is a double-stranded DNA molecule.

The two sequence homology regions are chosen according to the experimental design. There are no limitations on which regions of the first

DNA molecule can be chosen for the two sequence homology regions located on the second DNA molecule, except that the homologous recombination event cannot delete the origin of replication of the first DNA molecule. The sequence homology regions can be interrupted by non-identical sequence regions as long as sufficient sequence homology is retained for the homologous recombination reaction. By using sequence homology arms having non-identical sequence regions compared to the target site mutations such as substitutions, e.g. point mutations, insertions and/or deletions may be introduced into the target site by ET cloning.

The second foreign DNA molecule which is to be cloned in the bacterial cell may be derived from any source. For example, the second DNA molecule may be synthesized by a nucleic acid amplification reaction such as a PCR where both of the DNA oligonucleotides used to prime the amplification contain in addition to sequences at the 3′-ends that serve as a primer for the amplification, one or the other of the two homology regions. Using oligonucleotides of this design, the DNA product of the amplification can be any DNA sequence suitable for amplification and will additionally have a sequence homology region at each end.

A specific example of the generation of the second DNA molecule is the amplification of a gene that serves to convey a phenotypic difference to the bacterial host cells, in particular, antibiotic resistance. A simple variation of this procedure involves the use of oligonucleotides that include other sequences in addition to the PCR primer sequence and the sequence homology region. A further simple variation is the use of more than two amplification primers to generate the amplification product. A further simple variation is the use of more than one amplification reaction to generate the amplification product. A further variation is the use of DNA fragments obtained by methods other than PCR, for example, by endonuclease or restriction enzyme cleavage to linearize fragments from any source of DNA.

It should be noted that the second DNA molecule is not necessarily a single species of DNA molecule. It is of course possible to use a heterogenous population of second DNA molecules, e.g. to generate a DNA library, such as a genomic or cDNA library.

The method of the present invention may comprise the contacting of the first and second DNA molecules in vivo. In one embodiment of the present invention the second DNA fragment is transformed into a bacterial strain that already harbors the first vector DNA molecule. In a different embodiment, the second DNA molecule and the first DNA molecule are mixed together in vitro before co-transformation in the bacterial host cell. These two embodiments of the present invention are schematically depicted in Fig.1. The method of transformation can be any method known in the art (e.g. Sambrook et al. supra). The preferred method of transformation or co-transformation, however, is electroporation.

After contacting the first and second DNA molecules under conditions which favour homologous recombination between first and second DNA molecules via the ET cloning mechanism a host cell is selected, in which homologous recombination between said first and second DNA molecules has occurred. This selection procedure can be carried out by several different methods. In the following three preferred selection methods are depicted in Fig.2 and described in detail below.

In a first selection method a second DNA fragment is employed which carries a gene for a marker placed between the two regions of sequence homology wherein homologous recombination is detectable by expression of the marker gene. The marker gene may be a gene for a phenotypic marker which is not expressed in the host or from the first DNA molecule. Upon recombination by ET cloning, the change in phenotype of the host strain conveyed by the stable acquisition of the second DNA fragment identifies the ET cloning product.

In a preferred case, the phenotypic marker is a gene that conveys resistance to an antibiotic, in particular, genes that convey resistance to kanamycin, ampicillin, chloramphenicol, tetracyclin or any other substance that shows bacteriocidal or bacteriostatic effects on the bacterial strain employed.

A simple variation is the use of a gene that complements a deficiency present within the bacterial host strain employed. For example, the host strain may be mutated so that it is incapable of growth without a metabolic supplement. In the absence of this supplement, a gene on the second DNA fragment can complement the mutational defect thus permitting growth. Only those cells which contain the episome carrying the intended DNA rearrangement caused by the ET cloning step will grow.

In another example, the host strain carries a phenotypic marker gene which is mutated so that one of its codons is a stop codon that truncates the open reading frame. Expression of the full length protein from this phenotypic marker gene requires the introduction of a suppressor tRNA gene which, once expressed, recognizes the stop codon and permits translation of the full open reading frame. The suppressor tRNA gene is introduced by the ET cloning step and successful recombinants identified by selection for, or identification of, the expression of the phenotypic marker gene. In these cases, only those cells which contain the intended DNA rearrangement caused by the ET cloning step will grow.

A further simple variation is the use of a reporter gene that conveys a readily detectable change in colony colour or morphology. In a preferred case, the green fluorescence protein (GFP) can be used and colonies carrying the ET cloning product identified by the fluorescence emissions of GFP. In another preferred case, the lacZ gene can be used and colonies carrying the ET cloning product identified by a blue colony colour when X-gal is added to the culture medium.

In a second selection method the insertion of the second DNA fragment into the first DNA molecule by ET cloning alters the expression of a marker present on the first DNA molecule. In this embodiment the first DNA molecule contains at least one marker gene between the two regions of sequence homology and homologous recombination may be detected by an altered expression, e.g. lack of expression of the marker gene.

In a preferred application, the marker present on the first DNA molecule is a counter-selectable gene product, such as the sacB, ccdB or tetracycline-resistance genes. In these cases, bacterial cells that carry the first DNA molecule unmodified by the ET cloning step after transformation with the second DNA fragment, or co-transformation with the second DNA fragment and the first DNA molecule, are plated onto a medium so the expression of the counter-selectable marker conveys a toxic or bacteriostatic effect on the host. Only those bacterial cells which contain the first DNA molecule carrying the intended DNA rearrangement caused by the ET cloning step will grow.

In another preferred application, the first DNA molecule carries a reporter gene that conveys a readily detectable change in colony colour or morphology. In a preferred case, the green fluorescence protein (GFP) can be present on the first DNA molecule and colonies carrying the first DNA molecule with or without the ET cloning product can be distinguished by differences in the fluorescence emissions of GFP. In another preferred case, the lacZ gene can be present on the first DNA molecule and colonies carrying the first DNA molecule with or without the ET cloning product identified by a blue or white colony colour when X-gal is added to the culture medium.

In a third selection method the integration of the second DNA fragment into the first DNA molecule by ET cloning removes a target site for a site specific recombinase, termed here an RT (for recombinase target) present

on the first DNA molecule between the two regions of sequence homology. A homologous recombination event may be detected by removal of the target site.

In the absence of the ET cloning product, the RT is available for use by the corresponding site specific recombinase. The difference between the presence or not of this RT is the basis for selection of the ET cloning product. In the presence of this RT and the corresponding site specific recombinase, the site specific recombinase mediates recombination at this RT and changes the phenotype of the host so that it is either not able to grow or presents a readily observable phenotype. In the absence of this RT, the corresponding site specific recombinase is not able to mediate recombination.

In a preferred case, the first DNA molecule to which the second DNA fragment is directed, contains two RTs, one of which is adjacent to, but not part of, an antibiotic resistance gene. The second DNA fragment is directed, by design, to remove this RT. Upon exposure to the corresponding site specific recombinase, those first DNA molecules that do not carry the ET cloning product will be subject to a site specific recombination reaction between the RTs that remove the antibiotic resistance gene and therefore the first DNA molecule fails to convey resistance to the corresponding antibiotic. Only those first DNA molecules that contain the ET cloning product, or have failed to be site specifically recombined for some other reason, will convey resistance to the antibiotic.

In another preferred case, the RT to be removed by ET cloning of the second DNA fragment is adjacent to a gene that complements a deficiency present within the host strain employed. In another preferred case, the RT to be removed by ET cloning of the second DNA fragment is adjacent to a reporter gene that conveys a readily detectable change in colony colour or morphology.

In another preferred case, the RT to be removed by ET cloning of the second DNA fragment is anywhere on a first episomal DNA molecule and the episome carries an origin of replication incompatible with survival of the bacterial host cell if it is integrated into the host genome. In this case the host genome carries a second RT, which may or may not be a mutated RT so that the corresponding site specific recombinase can integrate the episome, via its RT, into the RT sited in the host genome. Other preferred RTs include RTs for site specific recombinases of the resolvase/transposase class. RTs include those described from existing examples of site specific recombination as well as natural or mutated variations thereof.

The preferred site specific recombinases include Cre, FLP, Kw or any site specific recombinase of the integrase class. Other preferred site specific recombinases include site specific recombinases of the resolvase/transposase class.

There are no limitations on the method of expression of the site specific recombinase in the host cell. In a preferred method, the expression of the site specific recombinase is regulated so that expression can be induced and quenched according to the optimisation of the ET cloning efficiency. In this case, the site specific recombinase gene can be either integrated into the host genome or carried on an episome. In another preferred case, the site specific recombinase is expressed from an episome that carries a conditional origin of replication so that it can be eliminated from the host cell.

In another preferred case, at least two of the above three selection methods are combined. A particularly preferred case involves a two-step use of the first selection method above, followed by use of the second selection method. This combined use requires, most simply, that the DNA fragment to be cloned includes a gene, or genes that permits the identification, in the first step, of correct ET cloning products by the acquisition of a phenotypic

change. In a second step, expression of the gene or genes introduced in the first step is altered so that a second round of ET cloning products can be identified. In a preferred example, the gene employed is the tetracycline resistance gene and the first step ET cloning products are identified by the acquisition of tetracycline resistance. In the second step, loss of expression of the tetracycline gene is identified by loss of sensitivity to nickel chloride, fusaric acid or any other agent that is toxic to the host cell when the tetracycline gene is expressed. This two-step procedure permits the identification of ET cloning products by first the integration of a gene that conveys a phenotypic change on the host, and second by the loss of a related phenotypic change, most simply by removal of some of the DNA sequences integrated in the first step. Thereby the genes used to identify ET cloning products can be inserted and then removed to leave ET cloning products that are free of these genes.

In a further embodiment of the present invention the ET cloning may also be used for a recombination method comprising the steps of

a) providing a source of RecE and RecT, or Redα and Redß, proteins,

b) contacting a first DNA molecule which is capable of being replicated in a suitable host cell with a second DNA molecule comprising at least two regions of sequence homology to regions on the first DNA molecule, under conditions which favour homologous recombination between said first and second DNA molecules and

c) selecting DNA molecules in which a homologous recombination between said first and second DNA molecules has occurred.

The source of RecE and RecT, or Redα and Redß, proteins may be either purified or partially purified RecE and RecT, or Redα and Redß, proteins or cell extracts comprising RecE and RecT, or Redα and Redß, proteins.

The homologous recombination event in this embodiment may occur in vitro, e.g. when providing a cell extract containing further components

required for homologous recombination. The homologous recombination event, however, may also occur in vivo, e.g. by introducing RecE and RecT, or Redα and Redß, proteins or the extract in a host cell (which may be recET positive or not, or redαß positive or not) and contacting the DNA molecules in the host cell. When the recombination occurs in vitro the selection of DNA molecules may be accomplished by transforming the recombination mixture in a suitable host cell and selecting for positive clones as described above. When the recombination occurs in vivo the selection methods as described above may directly be applied.

A further subject matter of the invention is the use of cells, preferably bacterial cells, most preferably, E.coli cells capable of expressing the recE and recT, or redα and redß, genes as a host cell for a cloning method involving homologous recombination.

Still a further subject matter of the invention is a vector system capable of expressing recE and recT, or redα and redß, genes in a host cell and its use for a cloning method involving homologous recombination. Preferably, the vector system is also capable of expressing an exonuclease inhibitor gene as defined above, e.g. the λ redγ gene. The vector system may comprise at least one vector. The recE and recT, or redα and redß, genes are preferably located on a single vector and more preferably under control of a regulatable promoter which may be the same for both genes or a single promoter for each gene. Especially preferred is a vector system which is capable of overexpressing the recT, or redß, gene versus the recE, or redα, gene.

Still a further subject matter of the invention is the use of a source of RecE and RecT, or Redα and Redß, proteins for a cloning method involving homologous recombination.

A still further subject matter of the invention is a reagent kit for cloning comprising

(a) a host cell, preferably a bacterial host cell,

(b) means of expressing recE and recT, or redα and redß, genes in said host cell, e.g. comprising a vector system, and

(c) a recipient cloning vehicle, e.g. a vector, capable of being replicated in said cell.

On the one hand, the recipient cloning vehicle which corresponds to the first DNA molecule of the process of the invention can already be present in the bacterial cell. On the other hand, it can be present separated from the bacterial cell.

In a further embodiment the reagent kit comprises

(a) a source for RecE and RecT, or Redα and Redß, proteins and

(b) a recipient cloning vehicle capable of being propagated in a host cell and

(c) optionally a host cell suitable for propagating said recipient cloning vehicle.

The reagent kit furthermore contains, preferably, means for expressing a site specific recombinase in said host cell, in particular, when the recipient ET cloning product contains at least one site specific recombinase target site. Moreover, the reagent kit can also contain DNA molecules suitable for use as a source of linear DNA fragments used for ET cloning, preferably by serving as templates for PCR generation of the linear fragment, also as specifically designed DNA vectors from which the linear DNA fragment is released by restriction enzyme cleavage, or as prepared linear fragments included in the kit for use as positive controls or other tasks. Moreover, the reagent kit can also contain nucleic acid amplification primers comprising a region of homology to said vector. Preferably, this region of homology is located at the 5'-end of the nucleic acid amplification primer.

The invention is further illustrated by the following Sequence listings, Figures and Examples.

SEQ ID NO. 1: shows the nucleic acid sequence of the plasmid pBAD24-rec ET (FIg. 7).

SEQ ID NOs 2/3: show the nucleic acid and amino acid sequences of the truncated recE gene (t-recE) present on pBAD24-recET at positions 1320-2162.

SEQ ID NOs 4/5: show the nucleic acid and amino acid sequences of the recT gene present on pBAD24-recET at position 2155-2972.

SEQ ID NOs 6/7: show the nucleic acid and amino acid sequences of the araC gene present on the complementary stand to the one shown of pBAD24-recET at positions 974-996.

SEQ ID NOs 8/9: show the nucleic acid an amino acid sequences of the bla gene present on pBAD24-recET at positions 3493-4353.

SEQ ID NO 10: shows the nucleic acid sequence of the plasmid pBAD-ETγ (Fig. 13).

SEQ ID No 11: shows the nucleic acid sequence of the plasmid pBAD-αßγ (Fig. 14) as well as the coding regions for the genes redα (1320-200), redß (2086-2871) and redγ (3403-3819).

SEQ ID NOs 12-14: show the amino acid sequences of the Redα, Redß and Redγ proteins, respectively. The redγ sequence is present on each of pBAD-ETγ (Fig. 13) and pBAD-αßγ (Fig. 14).

Figure 1

A preferred method for ET cloning is shown by diagram. The linear DNA fragment to be cloned is synthesized by PCR using oligonucleotide primers that contain a left homology arm chosen to match sequences in the recipient episome and a sequence for priming in the PCR reaction, and a

right homology arm chosen to match another sequence in the recipient episome and a sequence for priming in the PCR reaction. The product of the PCR reaction, here a selectable marker gene (sm1), is consequently flanked by the left and right homology arms and can be mixed together in vitro with the episome before co-transformation, or transformed into a host cell harboring the target episome. The host cell contains the products of the recE and recT genes. ET cloning products are identified by the combination of two selectable markers, sm1 and sm2 on the recipient episome.

Figure 2

Three ways to identify ET cloning products are depicted. The first, (on the left of the figure), shows the acquisition, by ET cloning, of a gene that conveys a phenotypic difference to the host, here a selectable marker gene (sm). The second (in the centre of the figure) shows the loss, by ET cloning, of a gene that conveys a phenotypic difference to the host, here a counter selectable marker gene (counter-sm). The third shows the loss of a target site (RT, shown as triangles on the circular episome) for a site specific recombinase (SSR), by ET cloning. In this case, the correct ET cloning product deletes one of the target sites required by the SSR to delete a selectable marker gene (sm). The failure of the SSR to delete the sm gene identifies the correct ET cloning product.

Figure 3

A simple example of ET cloning is presented.

(a) Top panel - PCR products (left lane) synthesized from oligonucleotides designed as described in Fig.1 to amplify by PCR a kanamycin resistance gene and to be flanked by homology arms present in the recipient vector, were mixed in vitro with the recipient vector (2nd lane) and cotransformed into a recET+ E.coli host. The recipient vector carried an ampillicin

resistance gene. (b) Transformation of the sbcA E.coli strain JC9604 with either the PCR product alone (0.2 μg) or the vector alone (0.3 μg) did not convey resistance to double selection with ampicillin and kanamycin (amp + kan), however cotransformation of both the PCR product and the vector produced double resistant colonies. More than 95% of these colonies contained the correct ET cloning product where the kanamycin gene had precisely integrated into the recipient vector according to the choice of homology arms. The two lanes on the right of (a) show Pvu II restriction enzyme digestion of the recipient vector before and after ET cloning. (c) As for b, except that six PCR products (0.2 μg each) were cotransformed with pSVpaZ11 (0.3 μg each) into JC9604 and plated onto Amp + Kan plates or Amp plates. Results are plotted as Amp + Kan-resistant colonies, representing recombination products, divided by Amp-resistant colonies, representing the plasmid transformation efficiency of the competent cell preparation, x 10^5. The PCR products were equivalent to the a-b PCR product except that homology arm lengths were varied. Results are from five experiments that used the same batches of competent cells and DNAs. Error bars represent standard deviation. (d) Eight products flanked by 50 bp homology arms were cotransformed with pSVpaZ11 into JC9604. All eight PCR products contained the same left homology arm and amplified neo gene. The right homology arms were chosen from the pSVpaZ11 sequence to be adjacent to (0), or at increasing distances (7-3100 bp), from the left. Results are from four experiments.

Figure 4

ET cloning in an approximately 100kb P1 vector to exchange the selectable marker.

A P1 clone which uses a kanamycin resistance gene as selectable marker and which contains at least 70kb of the mouse Hox a gene cluster was used. Before ET cloning, this episome conveys kanamycin resistance (top

panel, upper left) to its host E.coli which are ampillicin sensitive (top panel, upper right). A linear DNA fragment designed to replace the kanamycin resistance gene with an ampillicin resistance gene was made by PCR as outlined in Fig.1 and transformed into E.coli host cells in which the recipient Hox a/P1 vector was resident. ET cloning resulted in the deletion of the kanamycin resistance gene, and restoration of kanamycin sensitivity (top panel, lower left) and the acquisition of ampillicin resistance (top panel, lower right). Precise DNA recombination was verified by restriction digestion and Southern blotting analyses of isolated DNA before and after ET cloning (lower panel).

Figure 5

ET cloning to remove a counter selectable marker
A PCR fragment (upper panel, left, third lane) made as outlined in Figs.1 and 2 to contain the kanamycin resistance gene was directed by its chosen homology arms to delete the counter selectable ccdB gene present in the vector, pZero-2.1. The PCR product and the pZero vector were mixed in vitro (upper panel, left, 1st lane) before cotransformation into a recE/recT + E.coli host. Transformation of pZero-2.1 alone and plating onto kanamycin selection medium resulted in little colony growth (lower panel, left). Cotransformation of pZero-2.1 and the PCR product presented ET cloning products (lower panel, right) which showed the intended molecular event as visualized by Pvu II digestion (upper panel, right).

Figure 6

ET cloning mediated by inducible expression of recE and recT from an episome.
RecE/RecT mediate homologous recombination between linear and circular DNA molecules. (a) The plasmid pBAD24-recET was transformed into E.coli JC5547, and then batches of competent cells were prepared after induction of RecE/RecT expression by addition of L-arabinose for the times indicated

before harvesting. A PCR product, made using oligonucleotides e and f to contain the chloramphenicol resistance gene (cm) of pMAK705 and 50 bp homology arms chosen to flank the ampicillin resistance gene (bla) of pBAD24-recET, was then transformed and recombinants identified on chloramphenicol plates. (b) Arabinose was added to cultures of pBAD24-recET transformed JC5547 for different times immediately before harvesting for competent cell preparation. Total protein expression was analyzed by SDS-PAGE and Coomassie blue staining. (c) The number of chloramphenicol resistant colonies per μg of PCR product was normalized against a control for transformation efficiency, determined by including 5 pg pZero2.1, conveying kanamycin resistance, in the transformation and plating an aliquot onto Kan plates.

Figure 7A

The plasmid pBAD24-recET is shown by diagram. The plasmid contains the genes recE (in a truncated form) and recT under control of the inducible BAD promoter (P_{BAD}). The plasmid further contains an ampillicin resistance gene (Amp^r) and an araC gene.

Figure 7B

The nucleic acid sequence and the protein coding portions of pBAD24-recET are depicted.

Figure 8

Manipulation of a large E.coli episome by multiple recombination steps. a Scheme of the recombination reactions. A P1 clone of the Mouse Hoxa complex, resident in JC9604, was modified by recombination with PCR products that contained the neo gene and two Flp recombination targets

(FRTs). The two PCR products were identical except that one was flanked by g and h homology arms (insertion), and the other was flanked by i and h homology arms (deletion). In a second step, the neo gene was removed by Flp recombination between the FRTs by transient transformation of a Flp expression plasmid based on the pSC101 temperature-sensitive origin (ts ori). b Upper panel; ethidium bromide stained agarose gel showing EcoR1 digestions of P1 DNA preparations from three independent colonies for each step. Middle panel; a Southern blot of the upper panel hybridized with a neo gene probe. Lower panel: a Southern blot of the upper panel hybridized with a Hoxa3 probe to visualize the site of recombination. Lanes 1, the original Hoxa3 P1 clone grown in E.coli strain NS3145. Lanes 2, replacement of the Tn903 kanamycin resistance gene resident in the P1 vector with an ampicillin resistance gene increased the 8.1 kb band (lanes 1), to 9.0 kb. Lanes 3, insertion of the Tn5-neo gene with g-h homology arms upstream of Hoxa3, increased the 6.7 kb band (lanes 1,2) to 9.0 kb. Lanes 4, Flp recombinase deleted the g-h neo gene reducing the 9.0 kb band (lanes 3) back to 6.7 kb. Lanes 5, deletion of 6 kb of Hoxa3 - 4 intergenic DNA by replacement with the i-h neo gene, decreased the 6.7 kb band (lanes 2) to 4.5 kb. Lanes 6. Flp recombinase deleted the i-h neo gene reducing the 4.5 kb band to 2.3 kb.

Figure 9

Manipulation of the E.coli chromosome. A Scheme of the recombination reactions. The endogenous lacZ gene of JC9604 at 7.8' of the E.coli chromosome, shown in expanded form with relevant Ava I sites and coordinates, was targeted by a PCR fragment that contained the neo gene flanked by homology arms j and k, and loxP sites, as depicted. Integration of the neo gene removed most of the lacZ gene including an Ava I site to alter the 1443 and 3027 bp bands into a 3277 bp band. In a second step, the neo gene was removed by Cre recombination between the loxPs by transient transformation of a Cre expression plasmid based on the pSC101

temperature-sensitive origin (ts ori). Removal of the neo gene by Cre recombinase reduces the 3277 band to 2111 bp. b ß-galactosidase expression evaluated by streaking colonies on X-Gal plates. The top row of three streaks show ß-galactosidase expression in the host JC9604 strain (w.t.), the lower three rows (Km) show 24 independent primary colonies, 20 of which display a loss of ß-galactosidase expression indicactive of the intended recombination event. c Southern analysis of E.coli chromosomal DNA digested with Ava I using a random primed probe made from the entire lacZ coding region; lanes 1,2, w.t.; lanes 3-6, four independent white colonies after integration of the j-k neo gene; lanes 7-10; the same four colonies after transient transformation with the Cre expression plasmid.

Figure 10

Two rounds of ET cloning to introduce a point mutation. a Scheme of the recombination reactions. The lacZ gene of pSVpaX1 was disrupted in JC9604lacZ, a strain made by the experiment of Fig.9 to ablate endogenous lacZ expression and remove competitive sequences, by a sacB-neo gene cassette, synthesized by PCR to pIB279 and flanked by l and m homology arms. The recombinants, termed pSV-sacB-neo, were selected on Amp + Kan plates. The lacZ gene of pSV-sacB-neo was then repaired by a PCR fragment made from the intact lacZ gene using l* and m* homology arms. The m* homology arm included a silent C to G change that created a BamH1 site. The recombinants, termed pSVpaX1*, were identified by counter selection against the sacB gene using 7% sucrose. b ß-galactosidase expression from pSVpaX1 was disrupted in pSV-sacB-neo and restored in pSVpaX1*. Expression was analyzed on X-gal plates. Three independent colonies of each pSV-sacB-neo and pSVpaX1* are shown. c Ethidium bromide stained agarose gels of BamH1 digested DNA prepared from independent colonies taken after counter selection with sucrose. All ß-galactosidase expressing colonies (blue) contained the introduced BamH1 restriction site (upper panel). All white colonies displayed large

rearrangements and no product carried the diagnostic 1.5kb BamH1 restriction fragment (lower panel).

Figure 11

Transferance of ET cloning into a recBC + host to modify a large episome. a Scheme of the plasmid, pBAD-ETγ, which carries the mobile ET system, and the strategy employed to target the Hoxa P1 episome. pBAD-ETγ is based on pBAD24 and includes (i) the truncated recE gene (t-recE) under the arabinose-inducible P_{BAD} promoter; (ii) the recT gene under the EM7 promoter; and (iii) the redγ gene under the Tn5 promoter. It was transformed into NS3145, a recA E.coli strain which contained the Hoxa P1 episome. After arabinose induction, competent cells were prepared and transformed with a PCR product carrying the chloramphenicol resistance gene (cm) flanked by n and p homology arms. n and p were chosen to recombine with a segment of the P1 vector. b Southern blots of Pvu II digested DNAs hybridized with a probe made from the P1 vector to visualize the recombination target site (upper panel) and a probe made from the chloramphenicol resistance gene (lower panel). Lane 1, DNA prepared from cells harboring the Hoxa P1 episome before ET cloning. Lanes 2-17, DNA prepared from 16 independent chloramphenicol resistant colonies.

Figure 12

Comparison of ET cloning using the recE/recT genes in pBAD-ETγ with redα/redß genes in pBAD-αßγ.

The plasmids pBAD-ETγ or pBAD-αßγ, depicted, were transformed into the E.coli recA-, recBC + strain, DK1 and targeted by a chloramphenicol gene as described in Fig.6 to evaluate ET cloning efficiencies. Arabinose induction of protein expression was for 1 hour.

Figure 13A

The plasmid pBAD-ETγ is shown by diagram.

Figure 13B

The nucleic acid sequence and the protein coding portions of pBAD-ETγ are depicted.

Figure 14A

The plasmid pBAD-$\alpha\beta\gamma$ is shown by diagram. This plasmid substantially corresponds to the plasmid shown in Fig.13 except that the recE and recT genes are substituted by the redα and redβ genes.

Figure 14B

The nucleic acid sequence and the protein coding portions of pBAD-$\alpha\beta\gamma$ are depicted.

1. Methods

1.1. Preparation of linear fragments

Standard PCR reaction conditions were used to amplify linear DNA fragments. The sequences of the primers used are depicted in Table 1.

Table 1

The Tn5-neo gene from pJP5603 (Penfold and Pemberton, Gene 118 (1992), 145-146) was amplified by using oligo pairs a/b and c/d. The chloramphenicol (cm) resistant gene from pMAK705 (Hashimoto-Gotoh and

Sekiguchi, J.Bacteriol.131 (1977), 405-412) was amplified by using primer pairs e/f and n/p. The Tn5-neo gene flanked by FRT or loxP sites was amplified from pKaZ or pKaX (http://www.embl-heidelberg.de/ExternalInfo/stewart) using oligo pairs i/h, g/h and j/k. The sacB-neo cassette from pIB279 (Blomfield et al., Mol.Microbiol.5 (1991), 1447-1457) was amplified by using oligo pair l/m. The lacZ gene fragment from pSVpaZ11 (Buchholz et al., Nucleic Acids Res.24 (1996), 4256-4262) was amplified using oligo pair l'/m'. PCR products were purified using the QIAGEN PCR Purification Kit and eluted with H_2O_2, followed by digestion of any residual template DNA with Dpn I. After digestion, PCR products were extracted once with Phenol:$CHCl_3$, ethanol precipitated and resuspended in H_2O at approximately 0.5 μg/μl.

1.2 Preparation of competent cells and electroporation

Saturated overnight cultures were diluted 50 fold into LB medium, grown to an OD600 of 0.5, following by chilling on ice for 15 min. Bacterial cells were centrifuged at 7.000 rpm for 10 min at 0°C. The pellet was resuspended in ice-cold 10% glycerol and centrifuged again (7,000 rpm, -5°C, 10 min). This was repeated twice more and the cell pellet was suspended in an equal volume of ice-cold 10% glycerol. Aliquots of 50 μl were frozen in liquid nitrogen and stored at -80°C. Cells were thawed on ice and 1 μl DNA solution (containing, for co-transformation, 0.3 μg plasmid and 0.2 μg PCR products; or, for transformation, 0.2 μg PCR products) was added. Electroporation was performed using ice-cold cuvettes and a Bio-Rad Gene Pulser set to 25 μFD, 2.3 kV with Pulse Controller set at 200 ohms. LB medium (1 ml) was added after electroporation. The cells were incubated at 37°C for 1 hour with shaking and then spread on antibiotic plates.

1.3 Induction of RecE and RecT expression

E.coli JC5547 carrying pBAD24-recET was cultured overnight in LB medium plus 0.2% glucose, 100 μg/ml ampicillin. Five parallel LB cultures, one of which (O) included 0.2% glucose, were started by a 1/100 inoculation. The cultures were incubated at 37°C with shaking for 4 hours and 0.1% L-arabinose was added 3, 2, 1 or 1/2 hour before harvesting and processing as above. Immediately before harvesting, 100 μl was removed for analysis on a 10% SDS-polyacrylamide gel. E.coli NS3145 carrying Hoxa-P1 and pBAD-ETγ was induced by 0.1% L-arabinose for 90 min before harvesting.

1.4 Transient transformation of FLP and Cre expression plasmids

The FLP and Cre expression plasmids, 705-Cre and 705-FLP (Buchholz et al, Nucleic Acids Res. 24 (1996), 3118-3119), based on the pSC101 temperature sensitive origin, were transformed into rubidium chloride competent bacterial cells. Cells were spread on 25 μg/ml chloramphenicol plates, and grown for 2 days at 30°C, whereupon colonies were picked, replated on L-agar plates without any antibiotics and incubated at 40°C overnight. Single colonies were analyzed on various antibiotic plates and all showed the expected loss of chloramphenicol and kanamycin resistance.

1.5 Sucrose counter selection of sacB expression

The E.coli JC9604lacZ strain, generated as described in Fig.11, was cotransformed with a sacB-neo PCR fragment and pSVpaX1 (Buchholz et al, Nucleic Acids Res. 24 (1996), 4256-4262). After selection on 100 μg/ml ampicillin, 50 μg/ml kanamycin plates, pSVpaX-sacB-neo plasmids were isolated and cotransformed into fresh JC9604lacZ cells with a PCR fragment amplified from pSVpaX1 using primers l*/m*. Oligo m* carried a silent point mutation which generated a BamHI site. Cells were plated on 7% sucrose, 100 μg/ml ampicillin, 40 μg ml X-gal plates and incubated at

28°C for 2 days. The blue and white colonies grown on sucrose plates were counted and further checked by restriction analysis.

1.6 Other methods

DNA preparation and Southern analysis were performed according to standard procedures. Hybridization probes were generated by random priming of fragments isolated from the Tn5 neo gene (PvuII), Hoxa3 gene (both HindIII fragments), lacZ genes (EcoR1 and BamH1 fragments from pSVpaX1), cm gene (BstB1 fragments from pMAK705) and P1 vector fragments (2.2 kb EcoR1 fragments from P1 vector).

2. Results

2.1 Identification of recombination events in E.coli

To identify a flexible homologous recombination reaction in E.coli, an assay based on recombination between linear and circular DNAs was designed (Fig.1, Fig.3). Linear DNA carrying the Tn5 kanamycin resistance gene (neo) was made by PCR (Fig.3a). Initially, the oligonucleotides used for PCR amplification of neo were 60mers consisting of 42 nucleotides at their 5' ends identical to chosen regions in the plasmid and, at the 3' ends, 18 nucleotides to serve as PCR primers. Linear and circular DNAs were mixed in equimolar proportions and co-transformed into a variety of E.coli hosts. Homologous recombination was only detected in sbcA E.coli hosts. More than 95% of double ampicillin/kanamycin resistant colonies (Fig.3b) contained the expected homologously recombined plasmid as determined by restriction digestion and sequencing. Only a low background of kanamycin resistance, due to genomic integration of the neo gene, was apparent (not shown).

The linear plus circular recombination reaction was characterized in two ways. The relationship betweeen homology arm length and recombination efficiency was simple, with longer arms recombining more efficiently (Fig.3c). Efficiency increased within the range tested, up to 60 bp. The effect of distance between the two chosen homology sites in the recipient plasmid was examined (Fig.3d). A set of eight PCR fragments was generated by use of a constant left homology arm with differing right homology arms. The right homology arms were chosen from the plasmid sequence to be 0 - 3100 bp from the left. Correct products were readily obtained from all, with less than 4 fold difference between them, although the insertional product (0) was least efficient. Correct products also depended on the presence of both homology arms, since PCR fragments containing only one arm failed to work.

2.2 Involvement of RecE and RecT

The relationship between host genotype and this homologous recombination reaction was more systematically examined using a panel of E.coli strains deficient in various recombination components (Table 2).

Table 2

Only the two sbcA strains, JC8679 and JC9604 presented the intended recombination products and RecA was not required. In sbcA strains, expression of RecE and RecT is activated. Dependence on recE can be inferred from comparison of JC8679 with JC8691. Notably no recombination products were observed in JC9387 suggesting that the sbcBC background is not capable of supporting homologous recombination based on 50 nucleotide homology arms.

To demonstrate that RecE and RecT are involved, part of the recET operon was cloned into an inducible expression vector to create pBAD24-recET

(Fig.6a). the recE gene was truncated at its N-terminal end, as the first 588 a.a.s of RecE are dispensable. The recBC strain, JC5547, was transformed with pBAD24-recET and a time course of RecE/RecT induction performed by adding arabinose to the culture media at various times before harvesting for competent cells. The batches of harvested competent cells were evaluated for protein expression by gel electrophoresis (Fig.6b) and for recombination between a linear DNA fragment and the endogenous pBAD24-recET plasmid (Fig.6c). Without induction of RecE/RecT, no recombinant products were found, whereas recombination increased in approximate concordance with increased RecE/RecT expression. This experiment also shows that co-transformation of linear and circular DNAs is not essential and the circular recipient can be endogenous in the host. From the results shown in Figs.3, 6 and Table 2, we conclude that RecE and RecT mediate a very useful homologous recombination reaction in recBC E.coli at workable frequencies. Since RecE and RecT are involved, we refer to this way of recombining linear and circular DNA fragments as "ET cloning".

2.3 Application of ET cloning to large target DNAs

To show that large DNA episomes could be manipulated in E.coli, a > 76 kb P1 clone that contains at least 59 kb of the intact mouse Hoxa complex, (confirmed by DNA sequencing and Southern blotting), was transferred to an E.coli strain having an sbcA background (JC9604) and subjected to two rounds of ET cloning. In the first round, the Tn903 kanamycin resistance gene resident in the P1 vector was replaced by an ampicillin resistance gene (Fig.4). In the second round, the interval between the Hoxa3 and a4 genes was targeted either by inserting the neo gene between two base pairs upstream of the Hoxa3 proximal promoter, or by deleting 6203 bp between the Hoxa3 and a4 genes (Fig.8a). Both insertional and deletional ET cloning products were readily obtained (Fig.8b, lanes 2, 3 and 5) showing that the

two rounds of ET cloning took place in this large E.coli episome with precision and no apparent unintended recombination.

The general applicability of ET cloning was further examined by targeting a gene in the E.coli chromosome (Fig.9a). The ß-galactosidase (lacZ) gene of JC9604 was chosen so that the ratio between correct and incorrect recombinants could be determined by evaluating ß-galactosidase expression. Standard conditions (0.2 μg PCR fragment; 50 μl competent cells), produced 24 primary colonies, 20 of which were correct as determined by ß-galactosidase expression (Fig.9b), and DNA analysis (Fig.9c, lanes 3-6).

2.4 Secondary recombination reactions to remove operational sequences

The products of ET cloning as described above are limited by the necessary inclusion of selectable marker genes. Two different ways to use a further recombination step to remove this limitation were developed. In the first way, site specific recombination mediated by either Flp or Cre recombinase was employed. In the experiments of Figs.8 and 9, either Flp recombination target sites (FRTs) or Cre recombination target sites (loxPs) were included to flank the neo gene in the linear substrates. Recombination between the FRTs or loxPs was accomplished by Flp or Cre, respectively, expressed from plasmids with the pSC101 temperature sensitive replication origin (Hashimoto-Gotoh and Sekiguchi, J.Bacteriol. 131 (1977), 405-412) to permit simple elimination of these plasmids after site specific recombination by temperature shift. The precisely recombined Hoxa P1 vector was recovered after both ET and Flp recombination with no other recombination products apparent (Fig.8, lanes 4 and 6). Similarly, Cre recombinase precisely recombined the targeted lacZ allele (Fig.9, lanes 7-10). Thus site specific recombination can be readily coupled with ET cloning to remove operational sequences and leave a 34 bp site specific recombination target site at the point of DNA manipulation.

In the second way to remove the selectable marker gene, two rounds of ET cloning, combining positive and counter selection steps, were used to leave the DNA product free of any operational sequences (Fig.10a).

Additionally this experiment was designed to evaluate, by a functional test based on ß-galactosidase activity, whether ET cloning promoted small mutations such as frame shift or point mutations within the region being manipulated. In the first round, the lacZ gene of pSVpaX1 was disrupted with a 3.3 kb PCR fragment carrying the neo and B.subtilis sacB (Blomfield et al., Mol.Microbiol. 5 (1991), 1447-1457) genes, by selection for kanamycin resistance (Fig.10a). As shown above for other positively selected recombination products, virtually all selected colonies were white (Fig.10b), indicative of successful lacZ disruption, and 17 of 17 were confirmed as correct recombinants by DNA analysis. In the second round, a 1.5 kb PCR fragment designed to repair lacZ was introduced by counter selection against the sacB gene. Repair of lacZ included a silent point mutation to create a BamH1 restriction site. Approximately one quarter of sucrose resistant colonies expressed ß-galactosidase, and all analyzed (17 of 17; Fig.10c) carried the repaired lacZ gene with the BamH1 point mutation. The remaining three quarters of sucrose resistant colonies did not express ß-galactosidase, and all analyzed (17 of 17; Fig.10c) had undergone a variety of large mutational events, none of which resembled the ET cloning product. Thus, in two rounds of ET cloning directed at the lacZ gene, no disturbances of ß-galactosidase activity by small mutations were observed, indicating the RecE/RecT recombination works with high fidelity. The significant presence of incorrect products observed in the counter selection step is an inherent limitation of the use of counter selection, since any mutation that ablates expression of the counter selection gene will be selected. Notably, all incorrect products were large mutations and therefore easily distinguished from the correct ET product by DNA analysis. In a different experiment (Fig.5), we observed that ET cloning into pZero2.1 (InVitroGen) by counter selection against the ccdB gene gave

a lower background of incorrect products (8%), indicating that the counter selection background is variable according to parameters that differ from those that influence ET cloning efficiencies.

2.5 Transference of ET cloning between E.coli hosts

The experiments shown above were performed in recBC- E.coli hosts since the sbcA mutation had been identified as a suppressor of recBC (Barbour et al., Proc.Natl.Acad.Sci. USA 67 (1970), 128-135; Clark, Genetics 78 (1974), 259-271). However, many useful E.coli strains are recBC+, including strains commonly used for propagation of P1, BAC or PAC episomes. To transfer ET cloning into recBC+ strains, we developed pBAD-ETγ and pBAD-αßγ (Figs.13 and 14). These plasmids incorporate three features important to the mobility of ET cloning. First, RecBC is the major E.coli exonuclease and degrades introduced linear fragments. Therefore the RecBC inhibitor, Redγ (Murphy, J.Bacteriol. 173 (1991), 5808-5821), was included. Second, the recombinogenic potential of RecE/RecT, or Redα/Redß, was regulated by placing recE or redα under an inducible promoter. Consequently ET cloning can be induced when required and undesired recombination events which are restricted at other times. Third, we observed that ET cloning efficiencies are enhanced when RecT, or Redß, but not RecE, or Redα, is overexpressed. Therefore we placed recT, or redß, under the strong, constitutive, EM7 promoter.

pBAD-ETγ was transformed into NS3145 E.coli harboring the original Hoxa P1 episome (Fig.11a). A region in the P1 vector backbone was targeted by PCR amplification of the chloramphenicol resistance gene (cm) flanked by n and p homology arms. As described above for positively selected ET cloning reactions, most (> 90%) chloramphenicol resistant colonies were correct. Notably, the overall efficiency of ET cloning, in terms of linear DNA transformed, was nearly three times better using pBAD-ETγ than with similar experiments based on targeting the same episome in the sbcA host,

JC9604. This is consistent with our observation that overexpression of RecT improves ET cloning efficiencies.

A comparison between ET cloning efficiencies mediated by RecE/RecT, expressed from pBAD-ETγ, and Redα/Redß, expressed from pBAD-αßγ was made in the recA-, recBC+ E.coli strain, DK1 (Fig.12). After transformation of E.coli DK1 with either pBAD-ETγ or pBAD-αßγ, the same experiment as described in Figure 6a.c. to replace the bla gene of the pBAD vector with a chloramphenicol gene was performed. Both pBAD-ETγ or pBAD-αßγ presented similar ET cloning efficiencies in terms of responsiveness to arabinose induction of RecE and Redα, and number of targeted events.

Table 2

E.coli Strains	Genotypes	Amp+Kan	Amp
			x 10^8/µg
JC8679	*recBC sbcA*	318	2.30
JC9604	*recA recBC sbcA*	114	0.30
JC8691	*recBC sbcA recE*	0	0.37
JC5547	*recA recBC*	0	0.37
JC5519	*recBC*	0	1.80
JC15329	*recA recBC sbcBC*	0	0.03
JC9387	*recBC sbcBC*	0	2.20
JC8111	*recBC sbcBC recF*	0	2.40
JC9366	*recA*	0	0.37
JC13031	*recJ*	0	0.45

Claims

1. A method for cloning DNA molecules in cells comprising the steps of:

   a) providing a host cell capable of performing homologous recombination,

   b) contacting in said host cell a first DNA molecule which is capable of being replicated in said host cell with a second DNA molecule comprising at least two regions of sequence homology to regions on the first DNA molecule, under conditions which favour homologous recombination between said first and second DNA molecules and

   c) selecting a host cell in which homologous recombination between said first and second DNA molecules has occurred.

2. The method according to claim 1 wherein the homologous recombination occurs via the recET cloning mechanism.

3. The method according to claim 2 wherein the host cell is capable of expressing recE and recT genes.

4. The method according to claim 3 wherein the recE and recT genes are selected from E.coli recE and recT genes or from λ redα and redß genes.

5. The method according to claim 3 or 4 wherein the host cell is transformed with at least one vector capable of expressing recE and/or recT genes.

6. The method of claim 3, 4 or 5 wherein the expression of the recE and/or recT genes is under control of a regulatable promoter.

7. The method of claim 5 or 6 wherein the recT gene is overexpressed versus the recE gene.

8. The method according to any one of claims 3 to 7 wherein the recE gene is selected from a nucleic acid molecule comprising
(a) the nucleic acid sequence from position 1320 (ATG) to 2159 (GAC) as depicted in Fig.7B,
(b) the nucleic acid sequence from position 1320 (ATG) to 1998 (CGA) as depicted in Fig.13B,
(c) a nucleic acid encoding the same polypeptide within the degeneracy of the genetic code and/or
(d) a nucleic acid sequence which hybridizes under stringent conditions with the nucleic acid sequence from (a), (b) and/or (c).

9. The method according to any one of claims 3 to 8 wherein the recT gene is selected from a nucleic acid molecule comprising
(a) the nucleic acid sequence from position 2155 (ATG) to 2961 (GAA) as depicted in Fig.7B,
(b) the nucleic acid sequence from position 2086 (ATG) to 2868 (GCA) as depicted in Fig.13B,
(c) a nucleic acid encoding the same polypeptide within the degeneracy of the genetic code and/or
(d) a nucleic acid sequence which hybridizes under stringent conditions with the nucleic acid sequences from (a), (b) and/or (c).

10. The method according to any one of the previous claims wherein the host cell is a gram-negative bacterial cell.

11. The method according to claim 10 wherein the host cell is an Escherichia coli cell.

12. The method according to claim 11 wherein the host cell is an Escherichia coli K12 strain.

13. The method according to claim 12 wherein the E.coli strain is selected from JC 8679 and JC 9604.

14. The method according to any one of the previous claims wherein the host cell further is capable of expressing a recBC inhibitor gene.

15. The method according to claim 14 wherein the host cell is transformed with a vector expressing the recBC inhibitor gene.

16. The method according to claim 14 or 15 wherein the recBC inhibitor gene is selected from a nucleic acid molecule comprising
(a) the nucleic acid sequence from position 3588 (ATG) to 4002 (GTA) as depicted in Fig.13B,
(b) a nucleic acid encoding the same polypeptide within the degeneracy of the genetic code and/or
(c) a nucleic acid sequence which hybridizes under stringent conditions (as defined above) with the nucleic acid sequence from (a) and/ or (b).

17. The method according to any one of claims 13 to 16 wherein the host cell is a prokaryotic recBC+ cell.

18. The method according to any one of the previous claims wherein the first DNA molecule is circular.

19. The method according to any one of the previous claims wherein the first DNA molecule is an extrachromosomal DNA molecule containing an origin of replication which is operative in the host cell.

20. The method according to claim 18 or 19 wherein the first DNA molecule is selected from plasmids, cosmids, P1 vectors, BAC vectors and PAC vectors.

21. The method according to any one of claims 1-18 wherein the first DNA molecule is a host cell chromosome.

22. The method according to any one of the previous claims wherein the second DNA molecule is linear.

23. The method according to any one of the previous claims wherein the regions of sequence homology are at least 15 nucleotides each.

24. The method according to one of claims 1 to 16 wherein the second DNA molecule is obtained by an amplification reaction.

25. The method according to one of the previous claims wherein the first and/or second DNA molecules are introduced into the host cells by transformation.

26. The method according to claim 25 wherein the transformation method is electroporation.

27. The method according to one of claims 1 to 26 wherein the first and second DNA molecules are introduced into the host cell simultaneously by co-transformation.

28. The method according to one of claims 1 to 26 wherein the second DNA molecule is introduced into a host cell in which the first DNA molecule is already present.

43. A reagent kit for cloning comprising
(a) a host cell
(b) means of expressing recE and recT genes in said host cell and
(c) a recipient cloning vehicle capable of being replicated in said cell.

44. The reagent kit according to claim 43 wherein the means (b) comprise a vector system capable of expressing the recE and recT genes in the host cell.

45. The reagent kit according to claim 43 or 44 wherein the recE and recT genes are selected from E.coli recE and recT genes or from λ redα and redß genes.

46. A reagent kit for cloning comprising
(a) a source for RecE and RecT proteins and
(b) a recipient cloning vehicle capable of being propagated in a host cell.

47. The reagent kit according to claim 46 further comprising a host cell suitable for propagating said recipient cloning vehicle.

48. The reagent kit according to claim 46 or 47 wherein said RecE and RecT or proteins are selected from E.coli RecE and RecT proteins or from phage λ Redα and Redß proteins.

49. The reagent kit according to any one of claims 43-48 further comprising means for expressing a site specific recombinase in said host cell.

50. The reagent kit according to any one of claims 43-49 further comprising nucleic acid amplification primers comprising a region of homology to said recipient cloning vehicle.

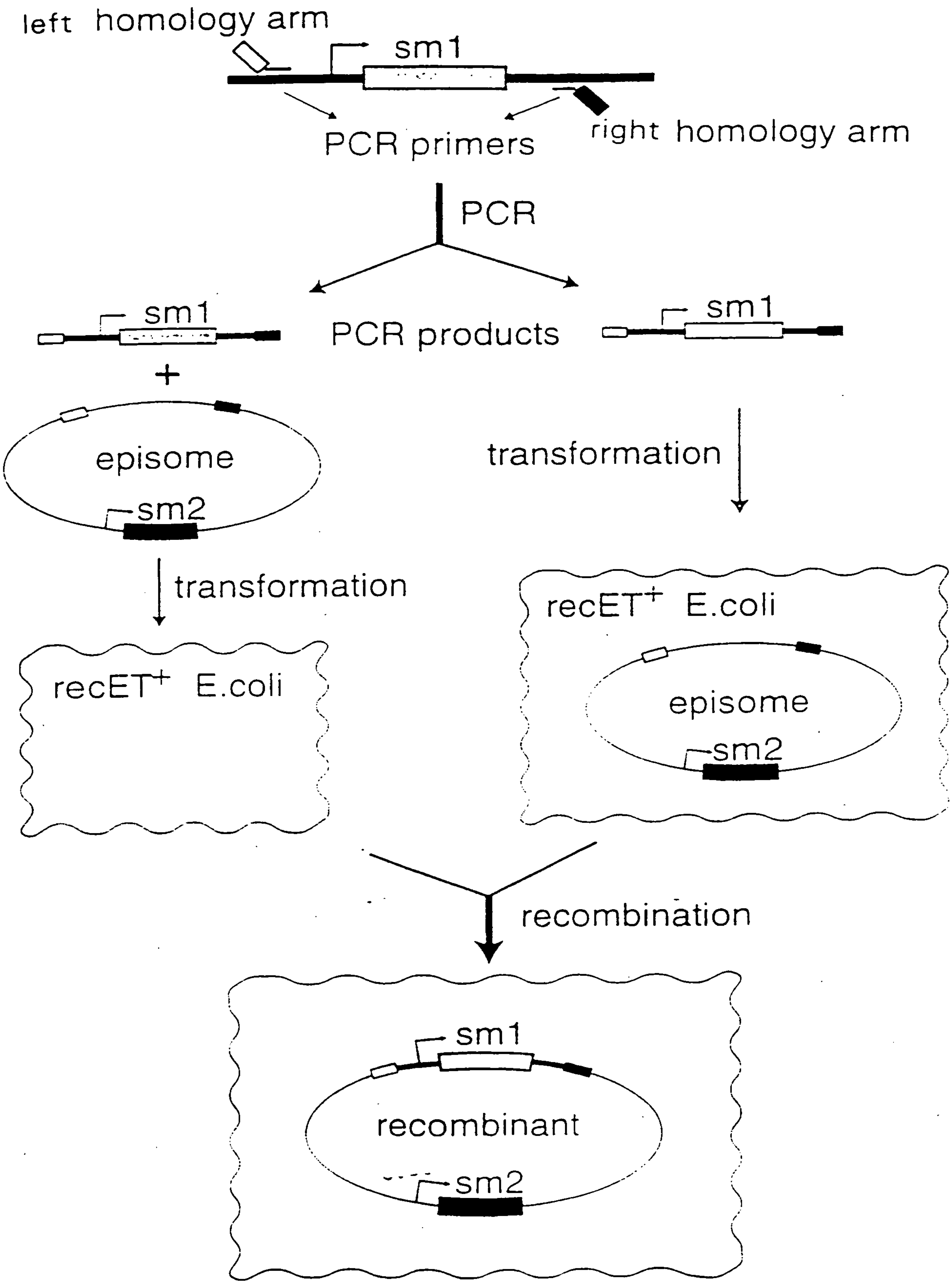

left homology arm
sm1
PCR primers
right homology arm
PCR
sm1
PCR products
sm1
+
episome
sm2
transformation
transformation
recET+ E.coli
recET+ E.coli
episome
sm2
recombination
sm1
recombinant
sm2

Figure 1

Three ways to select recombinants

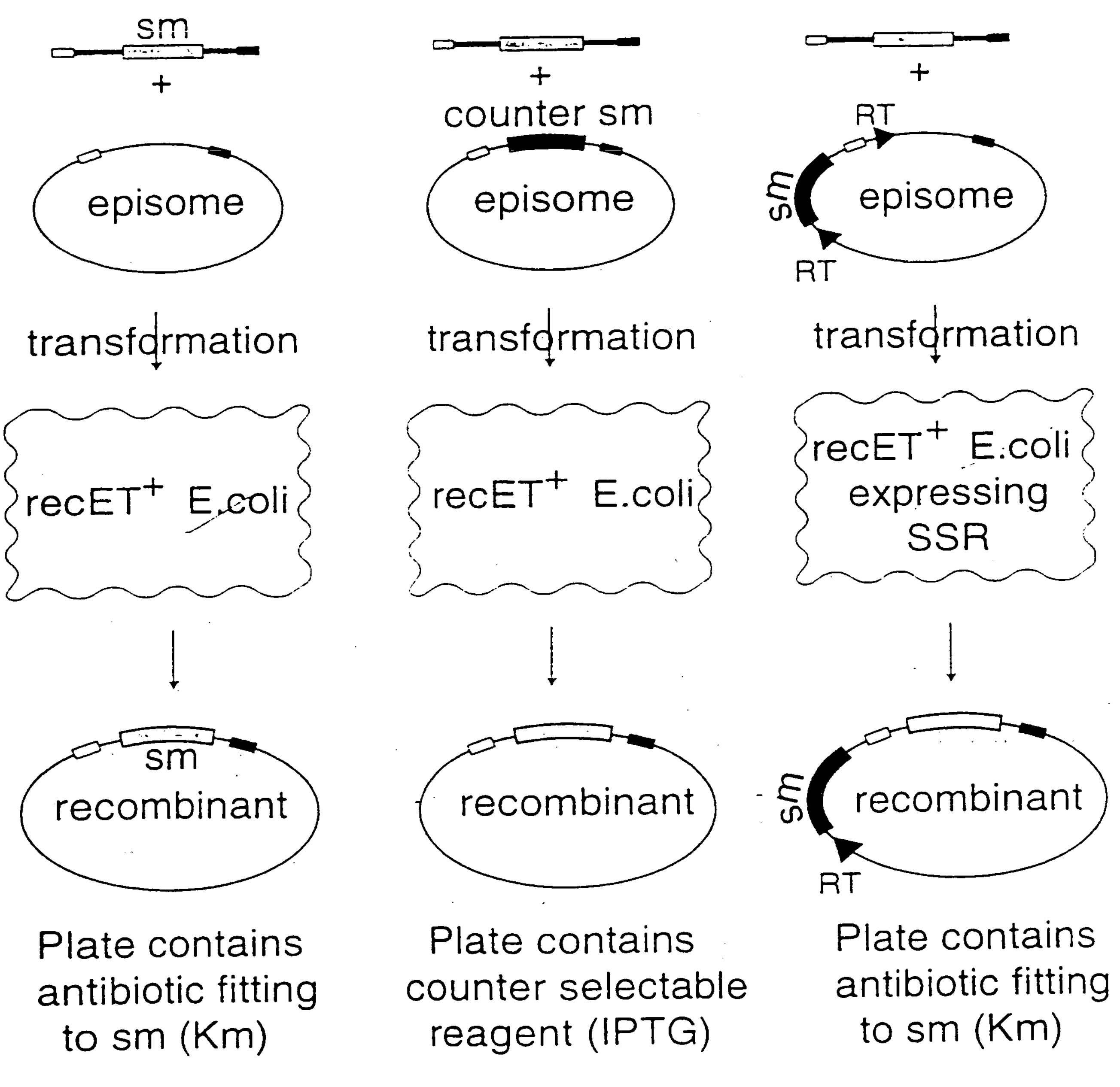
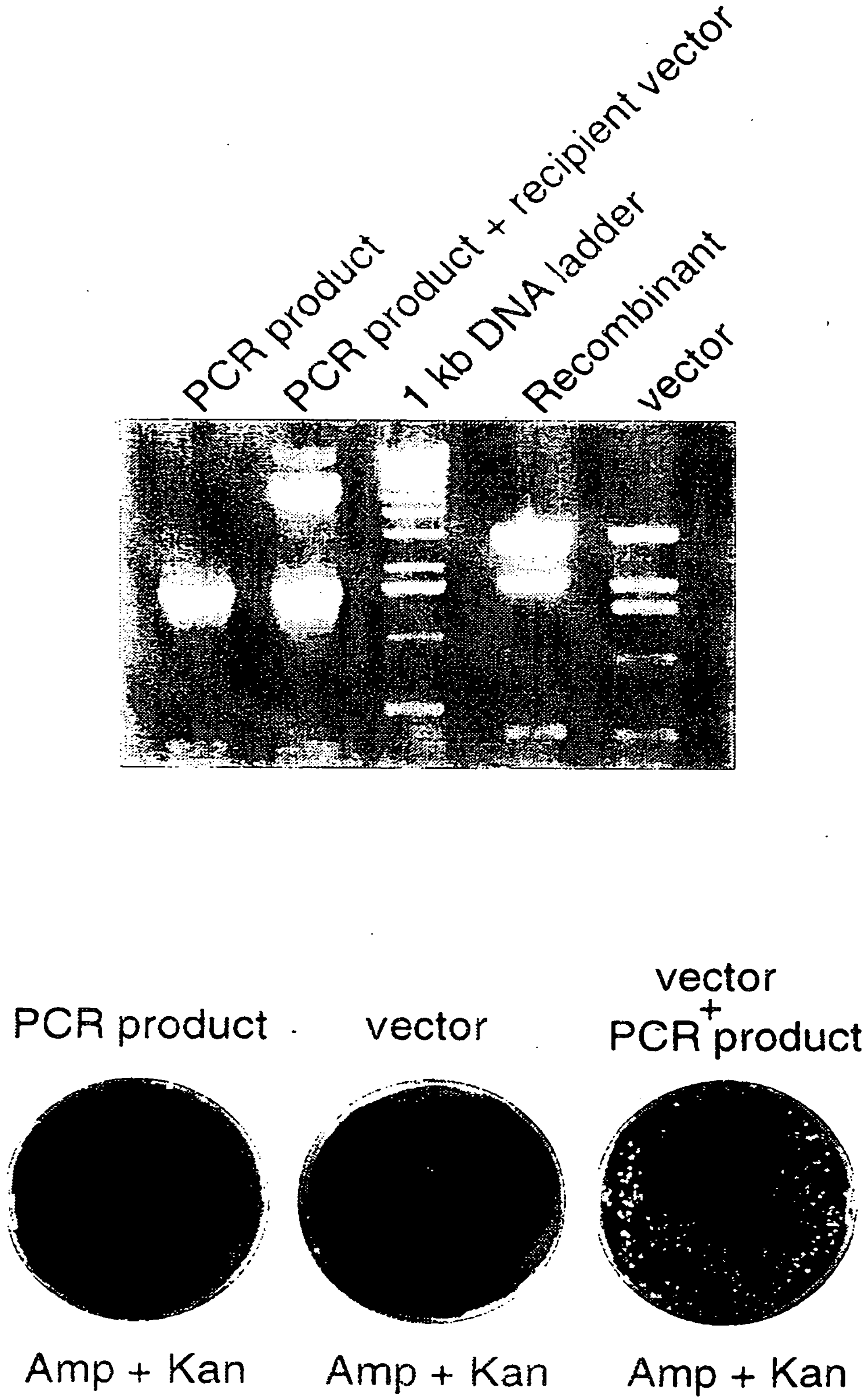


Figure 2

Figure 3

a

b


PCR product
PCR product + recipient vector
1 kb DNA ladder
Recombinant
vector
PCR product
vector
vector
+
PCR product
Amp + Kan
Amp + Kan
Amp + Kan

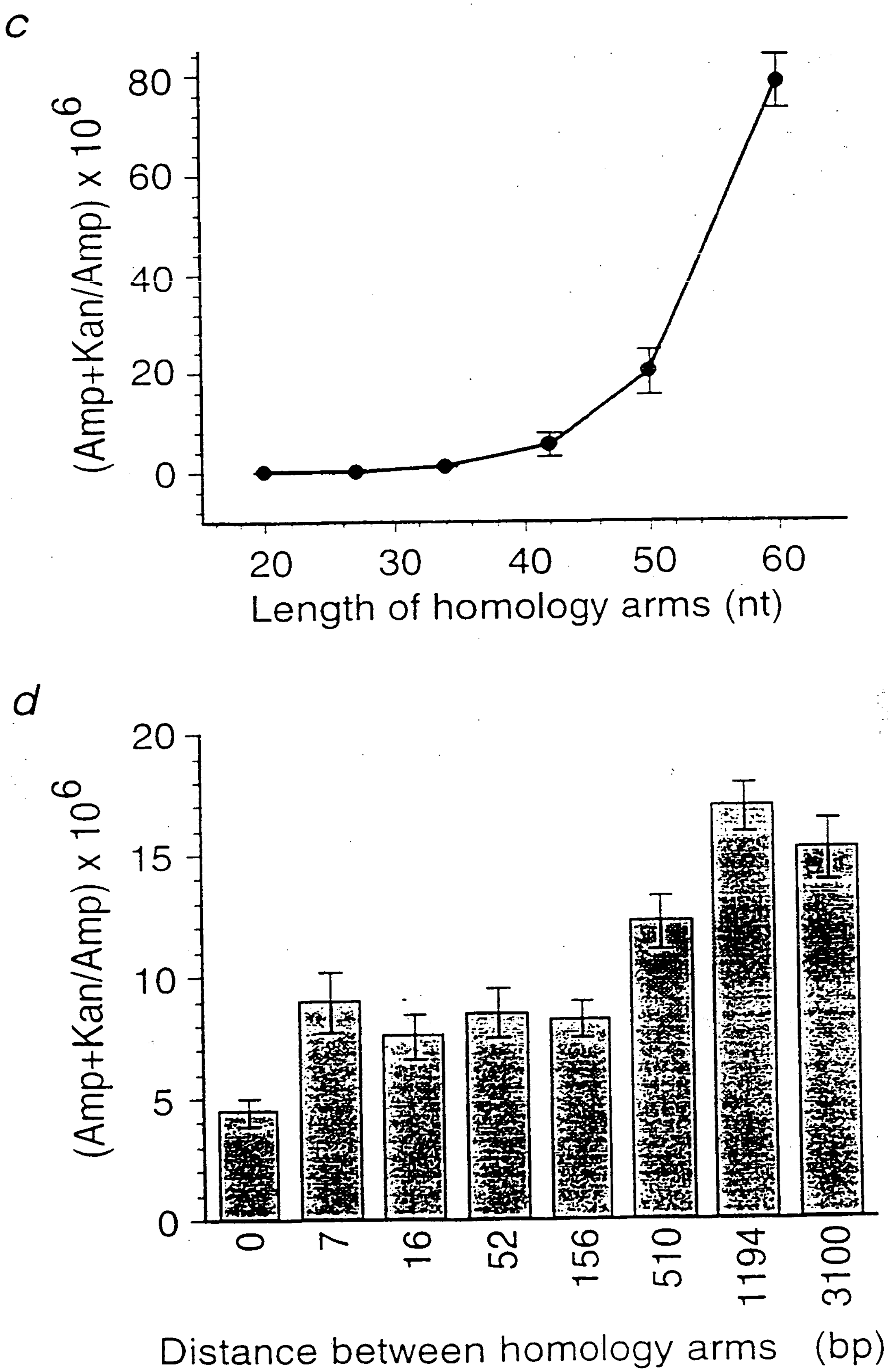

c
(Amp+Kan/Amp) x 10^6
80
60
40
20
0
20
30
40
50
60
Length of homology arms (nt)
d
(Amp+Kan/Amp) x 10^6
20
15
10
5
0
0
7
16
52
156
510
1194
3100
Distance between homology arms (bp)

Figure 3

Figure 4a

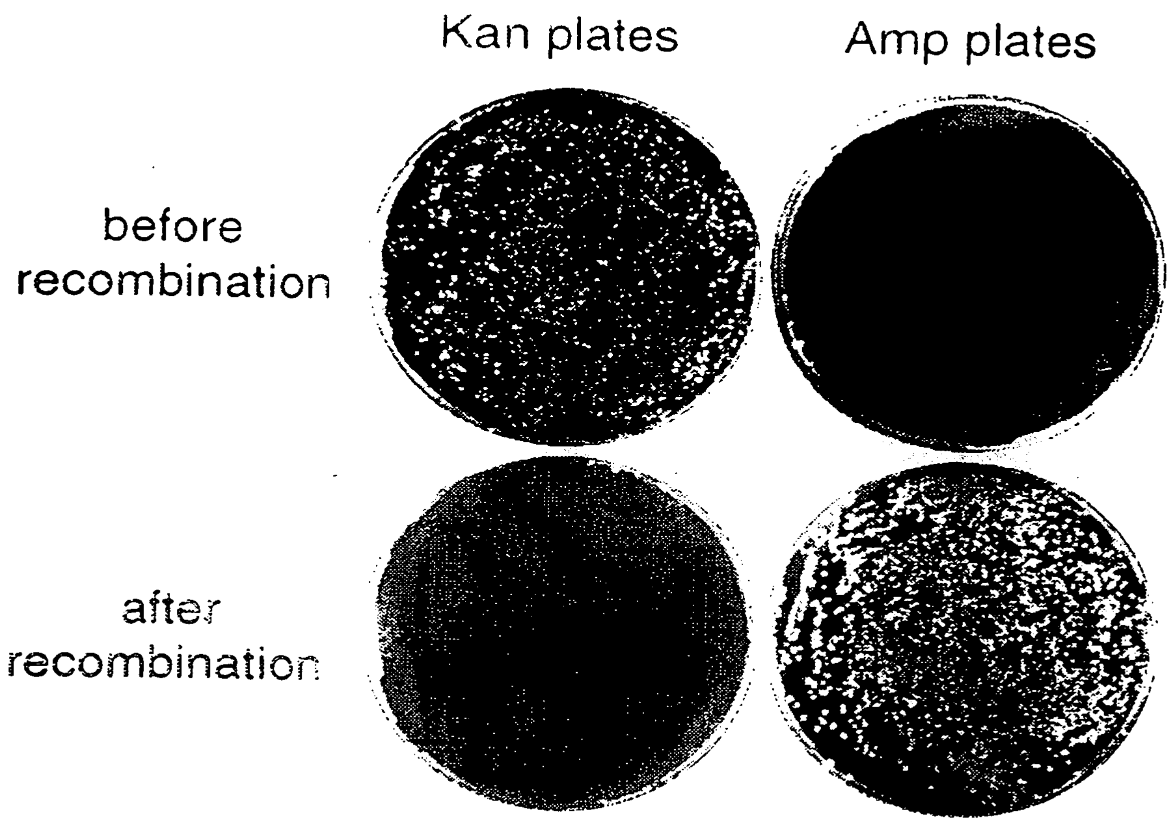
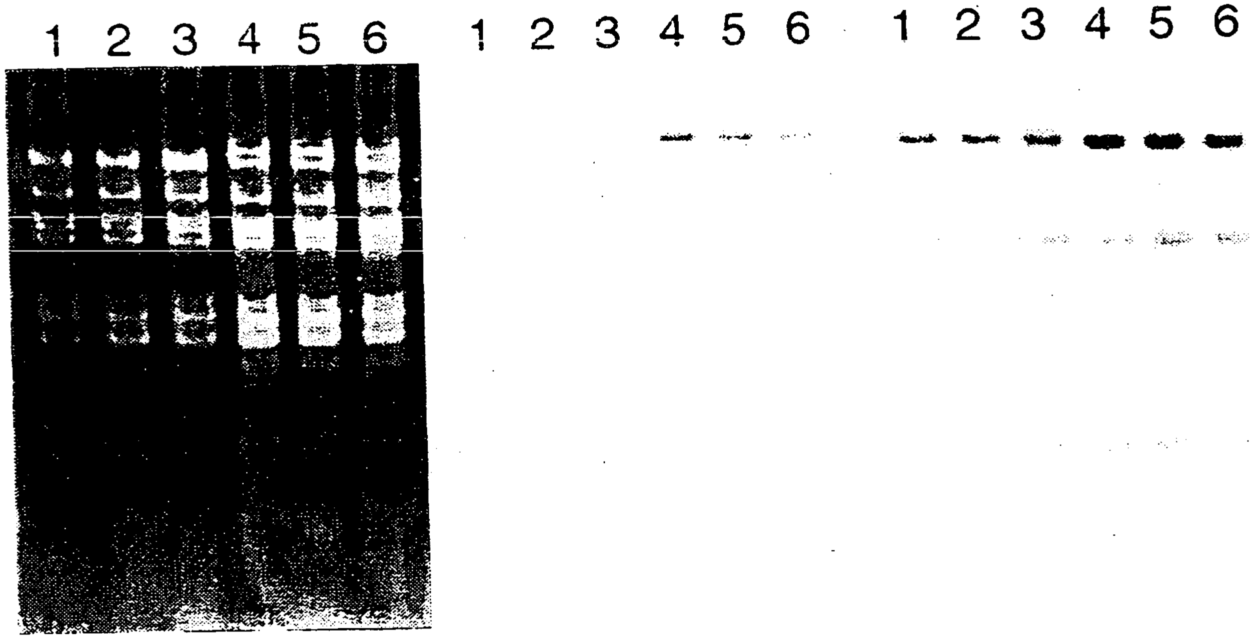

Kan plates
Amp plates
before recombination
after recombination

Figure 4b



P1 DNA digested with EcoR I

hybridized with a bla probe (Amp)

hybridized with a Hoxa-3 probe

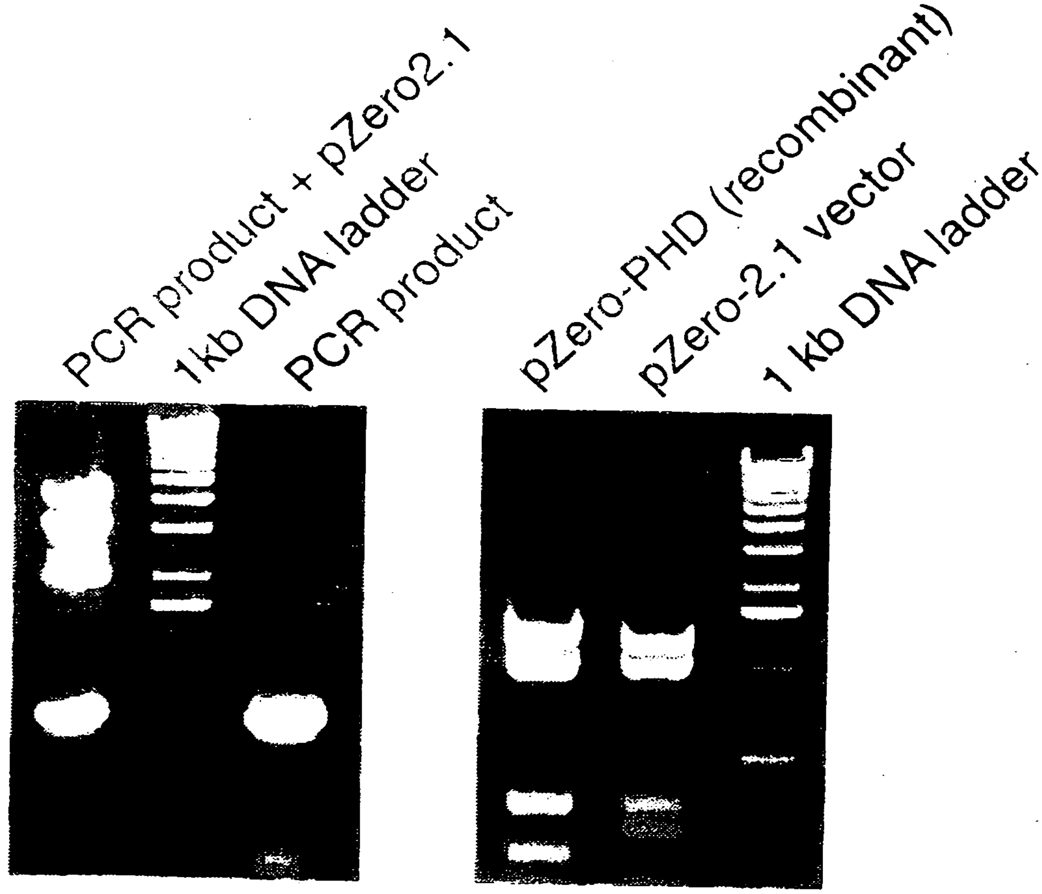
Lane 1: 1 of P1-Hox clone in NS3145 original bacterial strain (Kan resistance)

Lane 2-3: 2 of P1-Hox clones in JC9604 before homologous recombination (Kan resistance)

Lane 4-6: 3 of P1-Hox clones in JC9604 after homologous recombination (Amp resistance)

Figure 5

a


PCR product + pZero2.1
1kb DNA ladder
PCR product
pZero-PHD (recombinant)
pZero-2.1 vector
1 kb DNA ladder

b

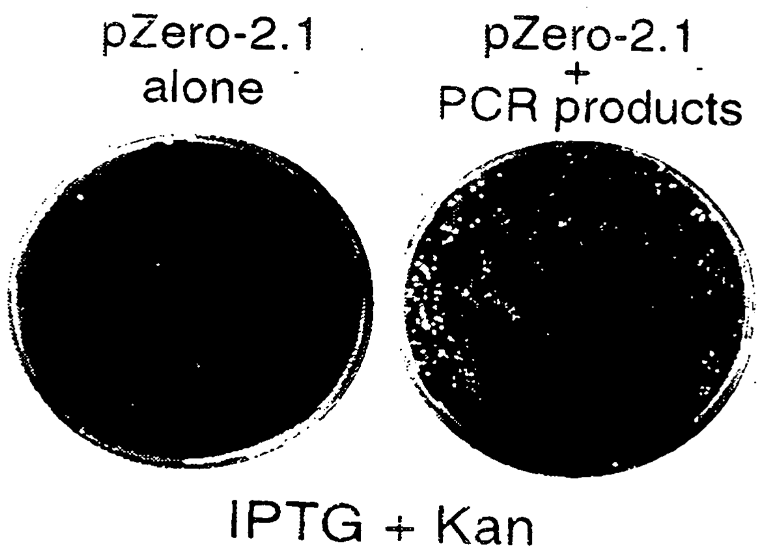

pZero-2.1
alone
pZero-2.1
+
PCR products
IPTG + Kan

Figure 6

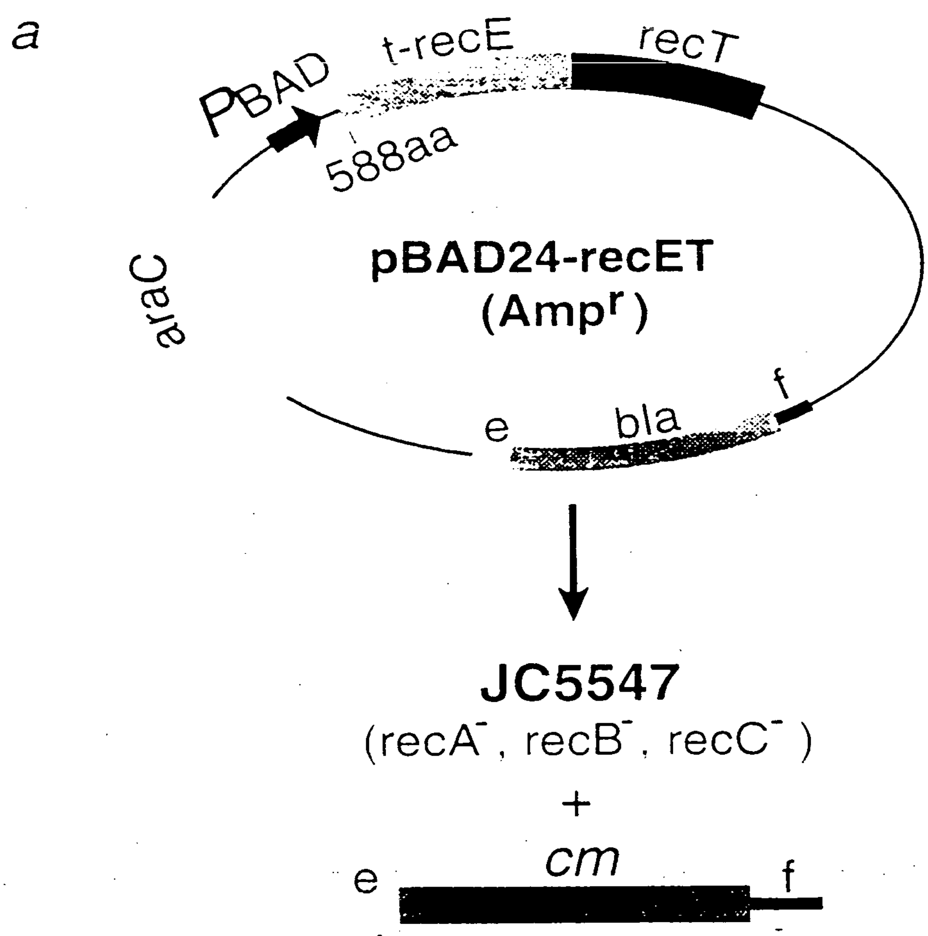
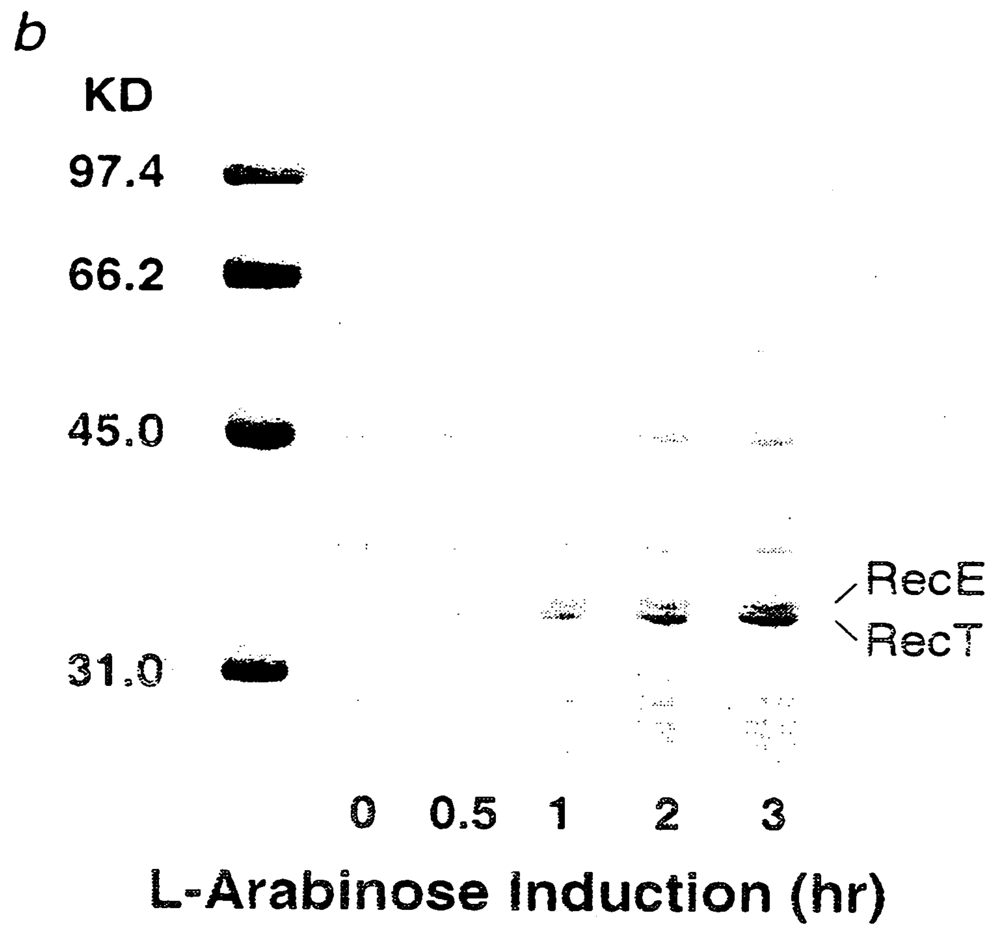
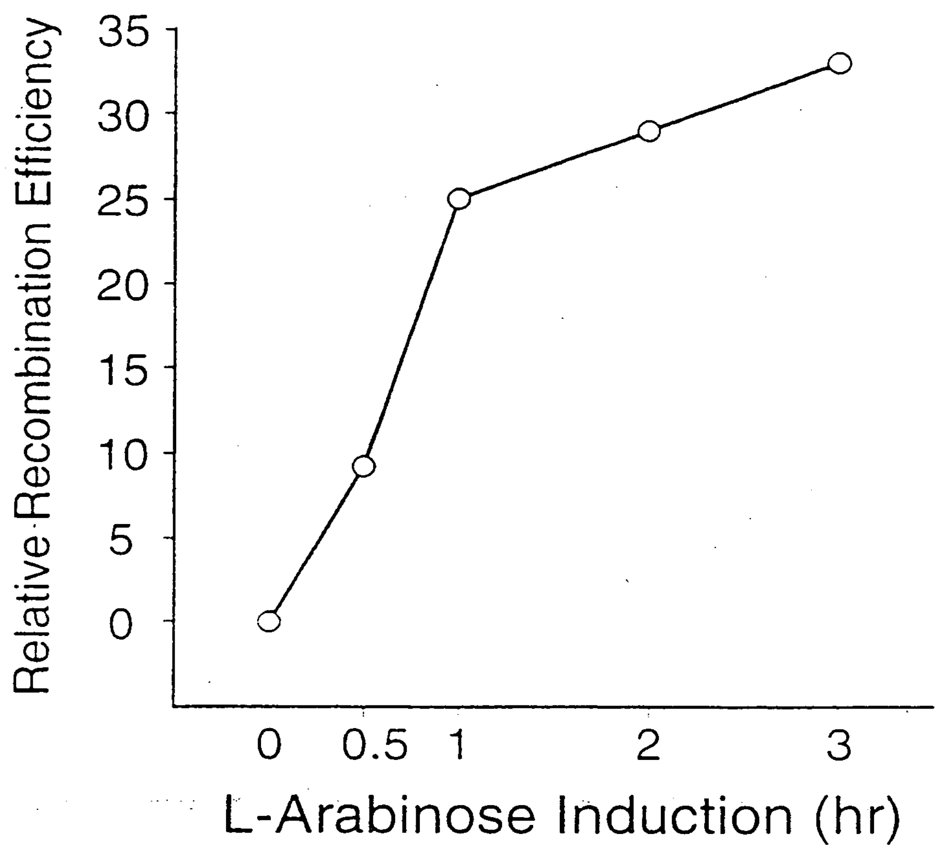

a
PBAD
t-recE
recT
588aa
araC
pBAD24-recET
(Amp^r)
e
bla
f
JC5547
(recA⁻, recB⁻, recC⁻)
+
e
cm
f

Figure 6


b
KD
97.4
66.2
45.0
31.0
RecE
RecT
0 0.5 1 2 3
L-Arabinose Induction (hr)

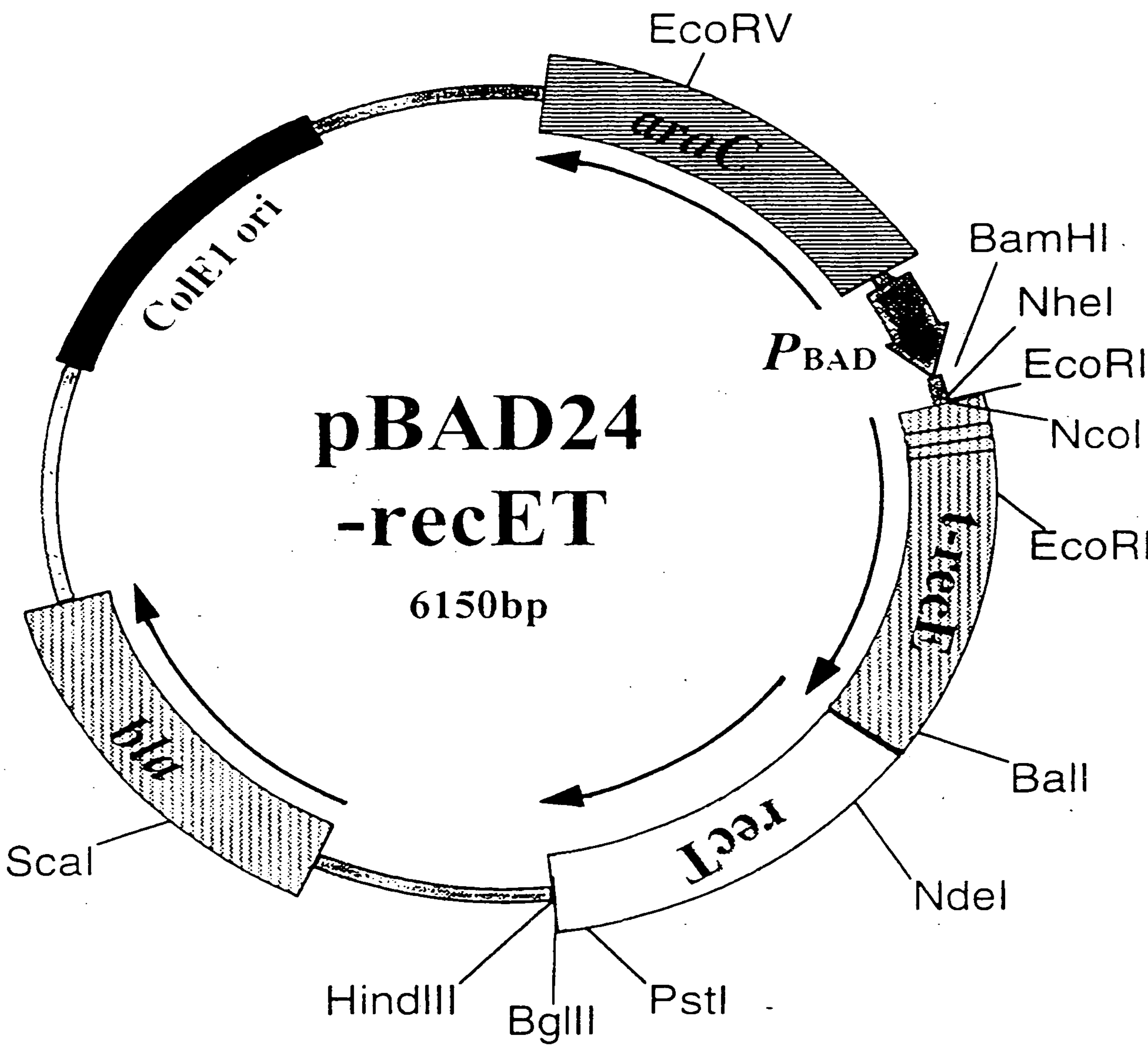
C



Relative Recombination Efficiency
35
30
25
20
15
10
5
0
0
0.5
1
2
3
L-Arabinose Induction (hr)

Figure 6

Figure 7a



t-recE --- truncated recE (from 588 aa ---> end. 866 aa)

Figure 7b

1 ATCGATGCATAATGTGCCTGTCAAATGGACGAAGCAGGGATTC
44 TGCAAACCCTATGCTACTCCGTCAAGCCGTCAATTGTCTGATT
87 CGTTACCAA TTA TGA CAA CTT GAC GGC TAC ATC
293◀• • • Ser Leu Lys Val Ala Val Asp

120 ATT CAC TTT TTC TTC ACA ACC GGC ACG GAA CTC
285◀Asn Val Lys Glu Glu Cys Gly Ala Arg Phe Glu

153 GCT CGG GCT GGC CCC GGT GCA TTT TTT AAA TAC
274◀Ser Pro Ser Ala Gly Thr Cys Lys Lys Phe Val

186 CCG CGA GAA ATA GAG TTG ATC GTC AAA ACC AAC
263◀Arg Ser Phe Tyr Leu Gln Asp Asp Phe Gly Val

219 ATT GCG ACC GAC GGT GGC GAT AGG CAT CCG GGT
252◀Asn Arg Gly Val Thr Ala Ile Pro Met Arg Thr

252 GGT GCT CAA AAG CAG CTT CGC CTG GCT GAT ACG
241◀Thr Ser Leu Leu Leu Lys Ala Gln Ser Ile Arg

285 TTG GTC CTC GCG CCA GCT TAA GAC GCT AAT CCC
230◀Gln Asp Glu Arg Trp Ser Leu Val Ser Ile Gly

318 TAA CTG CTG GCG GAA AAG ATG TGA CAG ACG CGA
219◀Leu Gln Gln Arg Phe Leu His Ser Leu Arg Ser

351 CGG CGA CAA GCA AAC ATG CTG TGC GAC GCT GGC
208◀Pro Ser Leu Cys Val His Gln Ala Val Ser Ala

EcoRV
384 GAT ATC AAA ATT GCT GTC TGC CAG GTG ATC GCT
197◀Ile Asp Phe Asn Ser Asp Ala Leu His Asp Ser

417 GAT GTA CTG ACA AGC CTC GCG TAC CCG ATT ATC
186◀Ile Tyr Gln Cys Ala Glu Arg Val Arg Asn Asp

Figure 7b (cont'd)

450	CAT	CGG	TGG	ATG	GAG	CGA	CTC	GTT	AAT	CGC	TTC
175◀	Met	Pro	Pro	His	Leu	Ser	Glu	Asn	Ile	Ala	Glu
483	CAT	GCG	CCG	CAG	TAA	CAA	TTG	CTC	AAG	CAG	ATT
164◀	Met	Arg	Arg	Leu	Leu	Leu	Gln	Glu	Leu	Leu	Asn
516	TAT	CGC	CAG	CAG	CTC	CGA	ATA	GCG	CCC	TTC	CCC
153◀	Ile	Ala	Leu	Leu	Glu	Ser	Tyr	Arg	Gly	Glu	Gly
549	TTG	CCC	GGC	GTT	AAT	GAT	TTG	CCC	AAA	CAG	GTC
142◀	Gln	Gly	Ala	Asn	Ile	Ile	Gln	Gly	Phe	Leu	Asp
582	GCT	GAA	ATG	CGG	CTG	GTG	CGC	TTC	ATC	CGG	GCG
131◀	Ser	Phe	His	Pro	Gln	His	Ala	Glu	Asp	Pro	Arg
615	AAA	GAA	CCC	CGT	ATT	GGC	AAA	TAT	TGA	CGG	CCA
120◀	Phe	Phe	Gly	Thr	Asn	Ala	Phe	Ile	Ser	Pro	Trp
648	GTT	AAG	CCA	TTC	ATG	CCA	GTA	GGC	GCG	CGG	ACG
109◀	Asn	Leu	Trp	Glu	His	Trp	Tyr	Ala	Arg	Pro	Arg
681	AAA	GTA	AAC	CCA	CTG	GTG	ATA	CCA	TTC	GCG	AGC
98◀	Phe	Tyr	Val	Trp	Gln	His	Tyr	Trp	Glu	Arg	Ala
714	CTC	CGG	ATG	ACG	ACC	GTA	GTG	ATG	AAT	CTC	TCC
87◀	Glu	Pro	His	Arg	Gly	Tyr	His	His	Ile	Glu	Gly
747	TGG	CGG	GAA	CAG	CAA	AAT	ATC	ACC	CGG	TCG	GCA
76◀	Pro	Pro	Phe	Leu	Leu	Ile	Asp	Gly	Pro	Arg	Cys
780	AAC	AAA	TTC	TCG	TCC	CTG	ATT	TTT	CAC	CAC	CCC
65◀	Val	Phe	Glu	Arg	Gly	Gln	Asn	Lys	Val	Val	Gly
813	CTG	ACC	GCG	AAT	GGT	GAG	ATT	GAG	AAT	ATA	ACC
54◀	Gln	Gly	Arg	Ile	Thr	Leu	Asn	Leu	Ile	Tyr	Gly
846	TTT	CAT	TCC	CAG	CGG	TCG	GTC	GAT	AAA	AAA	ATC
43◀	Lys	Met	Gly	Leu	Pro	Arg	Asp	Ile	Phe	Phe	Asp

Figure 7b (cont'd)

879 GAG ATA ACC GTT GGC CTC AAT CGG CGT TAA ACC
32◄Leu Tyr Gly Asn Ala Glu Ile Pro Thr Leu Gly

912 CGC CAC CAG ATG GGC ATT AAA CGA GTA TCC CGG
21◄Ala Val Leu His Ala Asn Phe Ser Tyr Gly Pro

945 CAG CAG GGG ATC ATT TTG CGC TTC AGC CAT
10◄Leu Leu Pro Asp Asn Gln Ala Glu Ala Met

975 ACTTTTCATA CTCCCGCCAT TCAGAGAAGA AACCAATTGT

1015 CCATATTGCA TCAGACATTG CCGTCACTGC GTCTTTTACT

1055 GGCTCTTCTC GCTAACCAAA CCGGTAACCC CGCTTATTAA

1095 AAGCATTCTG TAACAAAGCG GGACCAAAGC CATGACAAAA

1135 ACGCGTAACA AAAGTGTCTA TAATCACGGC AGAAAAGTCC

1175 ACATTGATTA TTTGCACGGC GTCACACTTT GCTATGCCAT

BamHI

1215 AGCATTTTTA TCCATAAGAT TAGCGGATCC TACCTGACGC

1255 TTTTTATCGC AACTCTCTAC TGTTTCTCCA TACCCGTTTT

Nhel EcoRI Ncol

1295 TTTGGGCTAG CAGGAGGAAT TCACC ATG GAT CCC GTA

1►Met Asp Pro Val

1332 ATC GTA GAA GAC ATA GAG CCA GGT ATT TAT TAC

5►Ile Val Glu Asp Ile Glu Pro Gly Ile Tyr Tyr

1365 GGA ATT TCG AAT GAG AAT TAC CAC GCG GGT CCC

16►Gly Ile Ser Asn Glu Asn Tyr His Ala Gly Pro

1398 GGT ATC AGT AAG TCT CAG CTC GAT GAC ATT GCT

Figure 7b (cont'd)

27▶ Gly Ile Ser Lys Ser Gln Leu Asp Asp Ile Ala

1431 GAT ACT CCG GCA CTA TAT TTG TGG CGT AAA AAT

38▶ Asp Thr Pro Ala Leu Tyr Leu Trp Arg Lys Asn

1464 GCC CCC GTG GAC ACC ACA AAG ACA AAA ACG CTC

49▶ Ala Pro Val Asp Thr Thr Lys Thr Lys Thr Leu

1497 GAT TTA GGA ACT GCT TTC CAC TGC CGG GTA CTT

60▶ Asp Leu Gly Thr Ala Phe His Cys Arg Val Leu

EcoRI

1530 GAA CCG GAA GAA TTC AGT AAC CGC TTT ATC GTA

71▶ Glu Pro Glu Glu Phe Ser Asn Arg Phe Ile Val

1563 GCA CCT GAA TTT AAC CGC CGT ACA AAC GCC GGA

82▶ Ala Pro Glu Phe Asn Arg Arg Thr Asn Ala Gly

1596 AAA GAA GAA GAG AAA GCG TTT CTG ATG GAA TGC

93▶ Lys Glu Glu Glu Lys Ala Phe Leu Met Glu Cys

1629 GCA AGC ACA GGA AAA ACG GTT ATC ACT GCG GAA

104▶ Ala Ser Thr Gly Lys Thr Val Ile Thr Ala Glu

1662 GAA GGC CGG AAA ATT GAA CTC ATG TAT CAA AGC

115▶ Glu Gly Arg Lys Ile Glu Leu Met Tyr Gln Ser

Figure 7b (cont'd)

1695 GTT ATG GCT TTG CCG CTG GGG CAA TGG CTT GTT

126▶ Val Met Ala Leu Pro Leu Gly Gln Trp Leu Val

1728 GAA AGC GCC GGA CAC GCT GAA TCA TCA ATT TAC

137▶ Glu Ser Ala Gly His Ala Glu Ser Ser Ile Tyr

1761 TGG GAA GAT CCT GAA ACA GGA ATT TTG TGT CGG

148▶ Trp Glu Asp Pro Glu Thr Gly Ile Leu Cys Arg

1794 TGC CGT CCG GAC AAA ATT ATC CCT GAA TTT CAC

159▶ Cys Arg Pro Asp Lys Ile Ile Pro Glu Phe His

1827 TGG ATC ATG GAC GTG AAA ACT ACG GCG GAT ATT

170▶ Trp Ile Met Asp Val Lys Thr Thr Ala Asp Ile

1860 CAA CGA TTC AAA ACC GCT TAT TAC GAC TAC CGC

181▶ Gln Arg Phe Lys Thr Ala Tyr Tyr Asp Tyr Arg

1893 TAT CAC GTT CAG GAT GCA TTC TAC AGT GAC GGT

192▶ Tyr His Val Gln Asp Ala Phe Tyr Ser Asp Gly

1926 TAT GAA GCA CAG TTT GGA GTG CAG CCA ACT TTC

203▶ Tyr Glu Ala Gln Phe Gly Val Gln Pro Thr Phe

1959 GTT TTT CTG GTT GCC AGC ACA ACT ATT GAA TGC

214▶ Val Phe Leu Val Ala Ser Thr Thr Ile Glu Cys

1992 GGA CGT TAT CCG GTT GAA ATT TTC ATG ATG GGC

Figure 7b (cont'd)

225▶Gly Arg Tyr Pro Val Glu Ile Phe Met Met Gly

2025 GAA GAA GCA AAA CTG GCA GGT CAA CAG GAA TAT

236▶Glu Glu Ala Lys Leu Ala Gly Gln Gln Glu Tyr

2058 CAC CGC AAT CTG CGA ACC CTG TCT GAC TGC CTG

247▶His Arg Asn Leu Arg Thr Leu Ser Asp Cys Leu

Ball

2091 AAT ACC GAT GAA TGG CCA GCT ATT AAG ACA TTA

258▶Asn Thr Asp Glu Trp Pro Ala Ile Lys Thr Leu

2124 TCA CTG CCC CGC TGG GCT AAG GAA TAT GCAA

269▶Ser Leu Pro Arg Trp Ala Lys Glu Tyr AlaA

2155 ATG ACT AAG CAA CCA CCA ATC GCA AAA GCC GAT

1▶Met Thr Lys Gln Pro Pro Ile Ala Lys Ala Asp

279▶s nAs p• • •

2188 CTG CAA AAA ACT CAG GGA AAC CGT GCA CCA GCA

12▶Leu Gln Lys Thr Gln Gly Asn Arg Ala Pro Ala

2221 GCA GTT AAA AAT AGC GAC GTG ATT AGT TTT ATT

23▶Ala Val Lys Asn Ser Asp Val Ile Ser Phe Ile

2254 AAC CAG CCA TCA ATG AAA GAG CAA CTG GCA GCA

34▶Asn Gln Pro Ser Met Lys Glu Gln Leu Ala Ala

NdeI

2287 GCT CTT CCA CGC CAT ATG ACG GCT GAA CGT ATG

45▶Ala Leu Pro Arg His Met Thr Ala Glu Arg Met

Figure 7b (cont'd)

2320	ATC	CGT	ATC	GCC	ACC	ACA	GAA	ATT	CGT	AAA	GTT
56▶	Ile	Arg	Ile	Ala	Thr	Thr	Glu	Ile	Arg	Lys	Val
2353	CCG	GCG	TTA	GGA	AAC	TGT	GAC	ACT	ATG	AGT	TTT
67▶	Pro	Ala	Leu	Gly	Asn	Cys	Asp	Thr	Met	Ser	Phe
2386	GTC	AGT	GCG	ATC	GTA	CAG	TGT	TCA	CAG	CTC	GGA
78▶	Val	Ser	Ala	Ile	Val	Gln	Cys	Ser	Gln	Leu	Gly
2419	CTT	GAG	CCA	GGT	AGC	GCC	CTC	GGT	CAT	GCA	TAT
89▶	Leu	Glu	Pro	Gly	Ser	Ala	Leu	Gly	His	Ala	Tyr
2452	TTA	CTG	CCT	TTT	GGT	AAT	AAA	AAC	GAA	AAG	AGC
100▶	Leu	Leu	Pro	Phe	Gly	Asn	Lys	Asn	Glu	Lys	Ser
2485	GGT	AAA	AAG	AAC	GTT	CAG	CTA	ATC	ATT	GGC	TAT
111▶	Gly	Lys	Lys	Asn	Val	Gln	Leu	Ile	Ile	Gly	Tyr
2518	CGC	GGC	ATG	ATT	GAT	CTG	GCT	CGC	CGT	TCT	GGT
122▶	Arg	Gly	Met	Ile	Asp	Leu	Ala	Arg	Arg	Ser	Gly
2551	CAA	ATC	GCC	AGC	CTG	TCA	GCC	CGT	GTT	GTC	CGT
133▶	Gln	Ile	Ala	Ser	Leu	Ser	Ala	Arg	Val	Val	Arg
2584	GAA	GGT	GAC	GAG	TTT	AGC	TTC	GAA	TTT	GGC	CTT
144▶	Glu	Gly	Asp	Glu	Phe	Ser	Phe	Glu	Phe	Gly	Leu
2617	GAT	GAA	AAG	TTA	ATA	CAC	CGC	CCG	GGA	GAA	AAC
155▶	Asp	Glu	Lys	Leu	Ile	His	Arg	Pro	Gly	Glu	Asn
2650	GAA	GAT	GCC	CCG	GTT	ACC	CAC	GTC	TAT	GCT	GTC
166▶	Glu	Asp	Ala	Pro	Val	Thr	His	Val	Tyr	Ala	Val
2683	GCA	AGA	CTG	AAA	GAC	GGA	GGT	ACT	CAG	TTT	GAA
177▶	Ala	Arg	Leu	Lys	Asp	Gly	Gly	Thr	Gln	Phe	Glu
2716	GTT	ATG	ACG	CGC	AAA	CAG	ATT	GAG	CTG	GTG	CGC
188▶	Val	Met	Thr	Arg	Lys	Gln	Ile	Glu	Leu	Val	Arg

Figure 7b (cont'd)

```
2749  AGC CTG AGT AAA GCT GGT AAT AAC GGG CCG TGG
 199▶ Ser Leu Ser Lys Ala Gly Asn Asn Gly Pro Trp

2782  GTA ACT CAC TGG GAA GAA ATG GCA AAG AAA ACG
 210▶ Val Thr His Trp Glu Glu Met Ala Lys Lys Thr

2815  GCT ATT CGT CGC CTG TTC AAA TAT TTG CCC GTA
 221▶ Ala Ile Arg Arg Leu Phe Lys Tyr Leu Pro Val

2848  TCA ATT GAG ATC CAG CGT GCA GTA TCA ATG GAT
 232▶ Ser Ile Glu Ile Gln Arg Ala Val Ser Met Asp
                                       PstI
2881  GAA AAG GAA CCA CTG ACA ATC GAT CCT GCA GAT
 243▶ Glu Lys Glu Pro Leu Thr Ile Asp Pro Ala Asp

2914  TCC TCT GTA TTA ACC GGG GAA TAC AGT GTA ATC
 254▶ Ser Ser Val Leu Thr Gly Glu Tyr Ser Val Ile
                             BglII     HindIII
2947  GAT AAT TCA GAG GAA TAG ATCTAAGCTT
 265▶ Asp Asn Ser Glu Glu •••

2975  GGCTGTTTTG GCGGATGAGA GAAGATTTTC AGCCTGATAC

3015  AGATTAAATC AGAACGCAGA AGCGGTCTGA TAAAACAGAA

3055  TTTGCCTGGC GGCAGTAGCG CGGTGGTCCC ACCTGACCCC

3095  ATGCCGAACT CAGAAGTGAA ACGCCGTAGC GCCGATGGTA

3135  GTGTGGGGTC TCCCCATGCG AGAGTAGGGA ACTGCCAGGC

3175  ATCAAATAAA ACGAAAGGCT CAGTCGAAAG ACTGGGCCTT

3215  TCGTTTTATC TGTTGTTTGT CGGTGAACGC TCTCCTGAGT

3255  AGGACAAATC CGCCGGGAGC GGATTTGAAC GTTGCGAAGC

3295  AACGGCCCGG AGGGTGGCGG GCAGGACGCC CGCCATAAAC

3335  TGCCAGGCAT CAAATTAAGC AGAAGGCCAT CCTGACGGAT
```

Figure 7b (cont'd)

```
3375  GGCCTTTTTG CGTTTCTACA AACTCTTTTG TTTATTTTTC
3415  TAAATACATT CAAATATGTA TCCGCTCATG AGACAATAAC
3455  CCTGATAAAT GCTTCAATAA TATTGAAAAA GGAAGAGT AT
                                            1▶Me
3495  G AGT ATT CAA CAT TTC CGT GTC GCC CTT ATT
   1▶t Ser Ile Gln His Phe Arg Val Ala Leu Ile
3526  CCC TTT TTT GCG GCA TTT TGC CTT CCT GTT TTT
  12▶Pro Phe Phe Ala Ala Phe Cys Leu Pro Val Phe
3559  GCT CAC CCA GAA ACG CTG GTG AAA GTA AAA GAT
  23▶Ala His Pro Glu Thr Leu Val Lys Val Lys Asp
3592  GCT GAA GAT CAG TTG GGT GCA CGA GTG GGT TAC
  34▶Ala Glu Asp Gln Leu Gly Ala Arg Val Gly Tyr
3625  ATC GAA CTG GAT CTC AAC AGC GGT AAG ATC CTT
  45▶Ile Glu Leu Asp Leu Asn Ser Gly Lys Ile Leu
3658  GAG AGT TTT CGC CCC GAA GAA CGT TTT CCA ATG
  56▶Glu Ser Phe Arg Pro Glu Glu Arg Phe Pro Met
3691  ATG AGC ACT TTT AAA GTT CTG CTA TGT GGC GCG
  67▶Met Ser Thr Phe Lys Val Leu Leu Cys Gly Ala
3724  GTA TTA TCC CGT GTT GAC GCC GGG CAA GAG CAA
  78▶Val Leu Ser Arg Val Asp Ala Gly Gln Glu Gln
3757  CTC GGT CGC CGC ATA CAC TAT TCT CAG AAT GAC
  89▶Leu Gly Arg Arg Ile His Tyr Ser Gln Asn Asp
                  ScaI
3790  TTG GTT GAG TAC TCA CCA GTC ACA GAA AAG CAT
 100▶Leu Val Glu Tyr Ser Pro Val Thr Glu Lys His
3823  CTT ACG GAT GGC ATG ACA GTA AGA GAA TTA TGC
 111▶Leu Thr Asp Gly Met Thr Val Arg Glu Leu Cys
```

Figure 7b (cont'd)

3856	AGT	GCT	GCC	ATA	ACC	ATG	AGT	GAT	AAC	ACT	GCG
122▶	Ser	Ala	Ala	Ile	Thr	Met	Ser	Asp	Asn	Thr	Ala
3889	GCC	AAC	TTA	CTT	CTG	ACA	ACG	ATC	GGA	GGA	CCG
133▶	Ala	Asn	Leu	Leu	Leu	Thr	Thr	Ile	Gly	Gly	Pro
3922	AAG	GAG	CTA	ACC	GCT	TTT	TTG	CAC	AAC	ATG	GGG
144▶	Lys	Glu	Leu	Thr	Ala	Phe	Leu	His	Asn	Met	Gly
3955	GAT	CAT	GTA	ACT	CGC	CTT	GAT	CGT	TGG	GAA	CCG
155▶	Asp	His	Val	Thr	Arg	Leu	Asp	Arg	Trp	Glu	Pro
3988	GAG	CTG	AAT	GAA	GCC	ATA	CCA	AAC	GAC	GAG	CGT
166▶	Glu	Leu	Asn	Glu	Ala	Ile	Pro	Asn	Asp	Glu	Arg
4021	GAC	ACC	ACG	ATG	CCT	GTA	GCA	ATG	GCA	ACA	ACG
177▶	Asp	Thr	Thr	Met	Pro	Val	Ala	Met	Ala	Thr	Thr
4054	TTG	CGC	AAA	CTA	TTA	ACT	GGC	GAA	CTA	CTT	ACT
188▶	Leu	Arg	Lys	Leu	Leu	Thr	Gly	Glu	Leu	Leu	Thr
4087	CTA	GCT	TCC	CGG	CAA	CAA	TTA	ATA	GAC	TGG	ATG
199▶	Leu	Ala	Ser	Arg	Gln	Gln	Leu	Ile	Asp	Trp	Met
4120	GAG	GCG	GAT	AAA	GTT	GCA	GGA	CCA	CTT	CTG	CGC
210▶	Glu	Ala	Asp	Lys	Val	Ala	Gly	Pro	Leu	Leu	Arg
4153	TCG	GCC	CTT	CCG	GCT	GGC	TGG	TTT	ATT	GCT	GAT
221▶	Ser	Ala	Leu	Pro	Ala	Gly	Trp	Phe	Ile	Ala	Asp
4186	AAA	TCT	GGA	GCC	GGT	GAG	CGT	GGG	TCT	CGC	GGT
232▶	Lys	Ser	Gly	Ala	Gly	Glu	Arg	Gly	Ser	Arg	Gly
4219	ATC	ATT	GCA	GCA	CTG	GGG	CCA	GAT	GGT	AAG	CCC
243▶	Ile	Ile	Ala	Ala	Leu	Gly	Pro	Asp	Gly	Lys	Pro
4252	TCC	CGT	ATC	GTA	GTT	ATC	TAC	ACG	ACG	GGG	AGT
254▶	Ser	Arg	Ile	Val	Val	Ile	Tyr	Thr	Thr	Gly	Ser

Figure 7b (cont'd)

4285 CAG GCA ACT ATG GAT GAA CGA AAT AGA CAG ATC
265▶ Gln Ala Thr Met Asp Glu Arg Asn Arg Gln Ile

4318 GCT GAG ATA GGT GCC TCA CTG ATT AAG CAT TGG
276▶ Ala Glu Ile Gly Ala Ser Leu Ile Lys His Trp

4351 TAA CTGTCAGACC AAGTTTACTC ATATATACTT
287▶ • • •

4384 TAGATTGATT TACGCGCCCT GTAGCGGCGC ATTAAGCGCG

4424 GCGGGTGTGG TGGTTACGCG CAGCGTGACC GCTACACTTG

4464 CCAGCGCCCT AGCGCCCGCT CCTTTCGCTT TCTTCCCTTC

4504 CTTTCTCGCC ACGTTCGCCG GCTTTCCCCG TCAAGCTCTA

4544 AATCGGGGGC TCCCTTTAGG GTTCCGATTT AGTGCTTTAC

4584 GGCACCTCGA CCCCAAAAAA CTTGATTTGG GTGATGGTTC

4624 ACGTAGTGGG CCATCGCCCT GATAGACGGT TTTTCGCCCT

4664 TTGACGTTGG AGTCCACGTT CTTTAATAGT GGACTCTTGT

4704 TCCAAACTGG AACAACACTC AACCCTATCT CGGGCTATTC

4744 TTTTGATTTA TAAGGGATTT TGCCGATTTC GGCCTATTGG

4784 TTAAAAAATG AGCTGATTTA ACAAAAATTT AACGCGAATT

4824 TTAACAAAAT ATTAACGTTT ACAATTTAAA AGGATCTAGG

4864 TGAAGATCCT TTTTGATAAT CTCATGACCA AAATCCCTTA

4904 ACGTGAGTTT TCGTTCCACT GAGCGTCAGA CCCCGTAGAA

4944 AAGATCAAAG GATCTTCTTG AGATCCTTTT TTTCTGCGCG

4984 TAATCTGCTG CTTGCAAACA AAAAAACCAC CGCTACCAGC

5024 GGTGGTTTGT TTGCCGGATC AAGAGCTACC AACTCTTTTT

Figure 7b (cont'd)

5064 CCGAAGGTAA CTGGCTTCAG CAGAGCGCAG ATACCAAATA
5104 CTGTCCTTCT AGTGTAGCCG TAGTTAGGCC ACCACTTCAA
5144 GAACTCTGTA GCACCGCCTA CATACCTCGC TCTGCTAATC
5184 CTGTTACCAG TGGCTGCTGC CAGTGGCGAT AAGTCGTGTC
5224 TTACCGGGTT GGACTCAAGA CGATAGTTAC CGGATAAGGC
5264 GCAGCGGTCG GGCTGAACGG GGGGTTCGTG CACACAGCCC
5304 AGCTTGGAGC GAACGACCTA CACCGAACTG AGATACCTAC
5344 AGCGTGAGCT ATGAGAAAGC GCCACGCTTC CCGAAGGGAG
5384 AAAGGCGGAC AGGTATCCGG TAAGCGGCAG GGTCGGAACA
5424 GGAGAGCGCA CGAGGGAGCT TCCAGGGGGA AACGCCTGGT
5464 ATCTTTATAG TCCTGTCGGG TTTCGCCACC TCTGACTTGA
5504 GCGTCGATTT TTGTGATGCT CGTCAGGGGG GCGGAGCCTA
5544 TGGAAAAACG CCAGCAACGC GGCCTTTTTA CGGTTCCTGG
5584 CCTTTTGCTG GCCTTTTGCT CACATGTTCT TTCCTGCGTT
5624 ATCCCCTGAT TCTGTGGATA ACCGTATTAC CGCCTTTGAG
5664 TGAGCTGATA CCGCTCGCCG CAGCCGAACG ACCGAGCGCA
5704 GCGAGTCAGT GAGCGAGGAA GCGGAAGAGC GCCTGATGCG
5744 GTATTTTCTC CTTACGCATC TGTGCGGTAT TTCACACCGC
5784 ATAGGGTCAT GGCTGCGCCC CGACACCCGC CAACACCCGC
5824 TGACGCGCCC TGACGGGCTT GTCTGCTCCC GGCATCCGCT
5864 TACAGACAAG CTGTGACCGT CTCCGGGAGC TGCATGTGTC
5904 AGAGGTTTTC ACCGTCATCA CCGAAACGCG CGAGGCAGCA
5944 AGGAGATGGC GCCCAACAGT CCCCCGGCCA CGGGGCCTGC

Figure 7b (cont'd)

5984 CACCATACCC ACGCCGAAAC AAGCGCTCAT GAGCCCGAAG
6024 TGGCGAGCCC GATCTTCCCC ATCGGTGATG TCGGCGATAT
6064 AGGCGCCAGC AACCGCACCT GTGGCGCCGG TGATGCCGGC
6104 CACGATGCGT CCGGCGTAGA GGATCTGCTC ATGTTTGACA
6144 GCTTATC

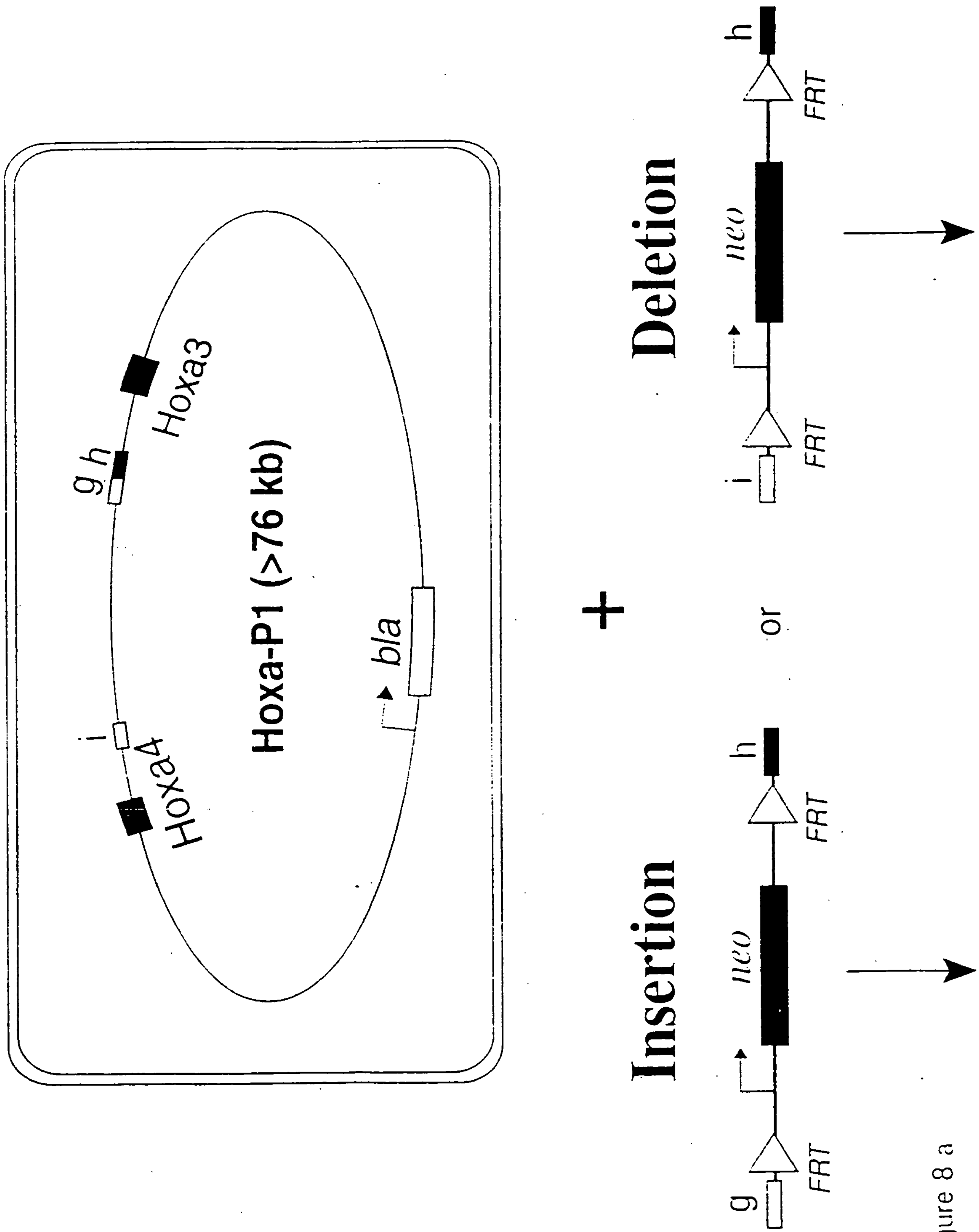

Hoxa-P1 (>76 kb)
Hoxa3
Hoxa4
g h
i
bla
+
Deletion
Insertion
or
neo
FRT
g
h
i

Figure 8 a

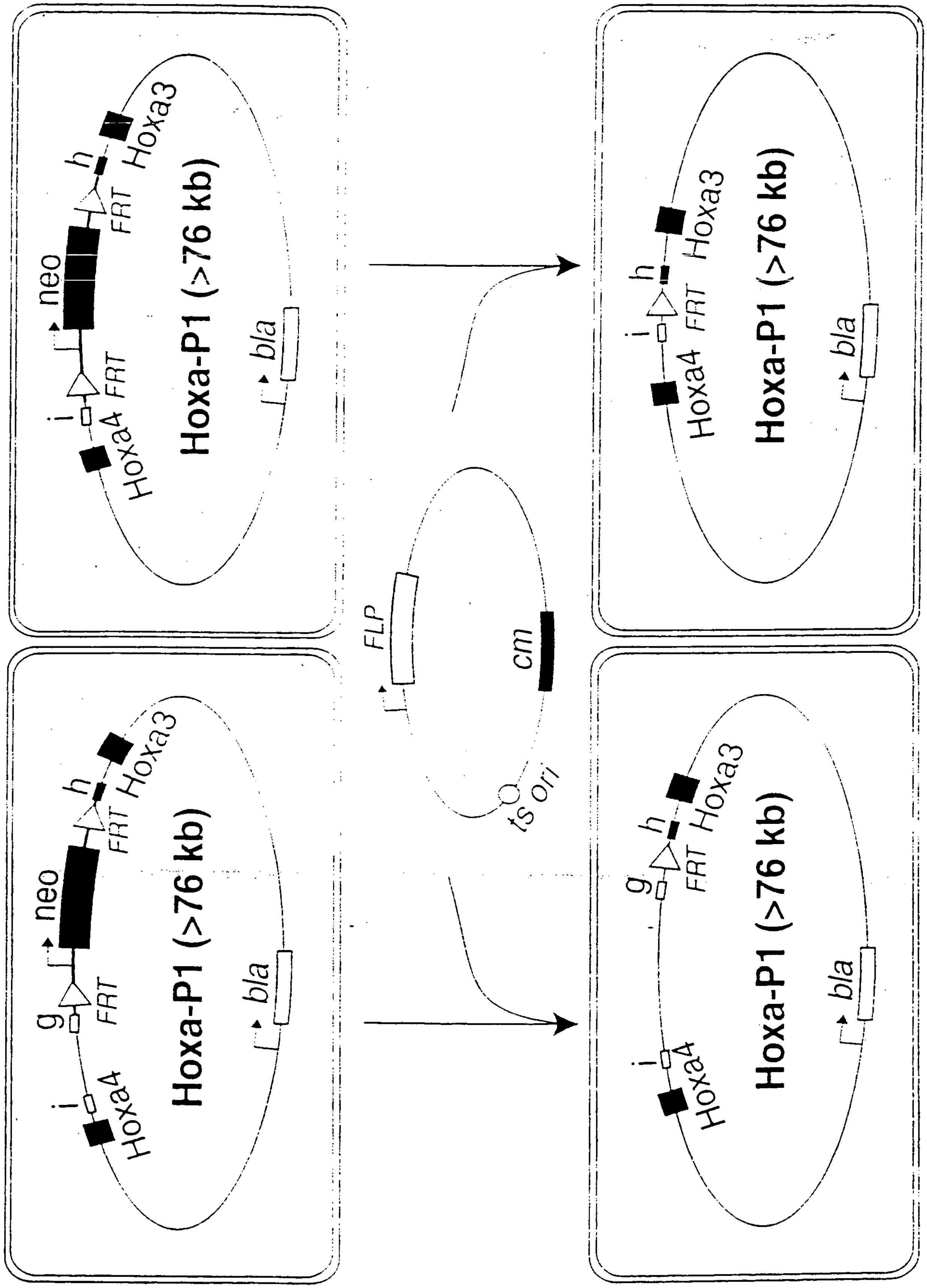


Figure 8 a (continuing)

Figure 8 b

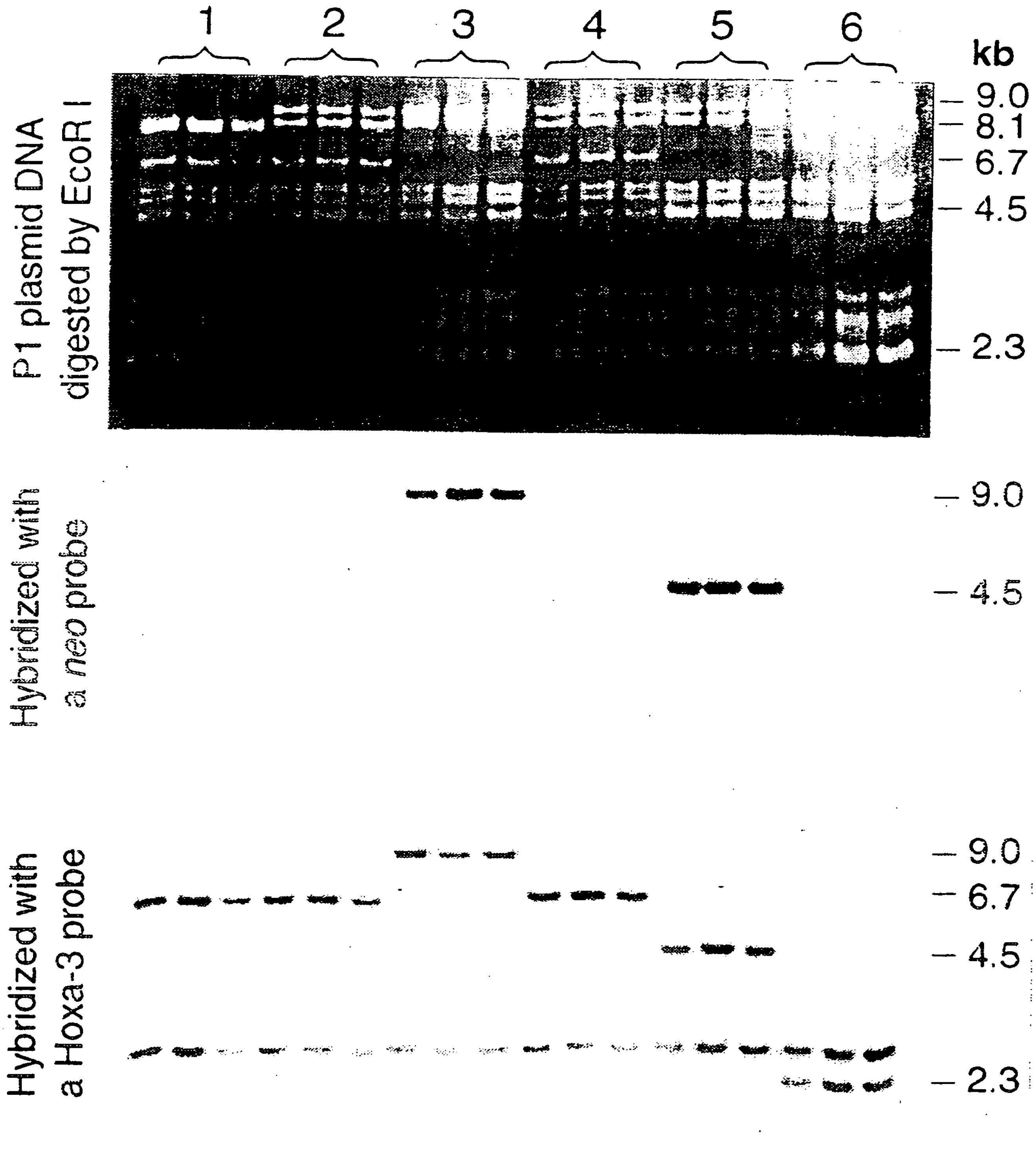

1
2
3
4
5
6
kb
P1 plasmid DNA digested by EcoR I
9.0
8.1
6.7
4.5
2.3
Hybridized with a *neo* probe
9.0
4.5
Hybridized with a Hoxa-3 probe
9.0
6.7
4.5
2.3

Figure 9a

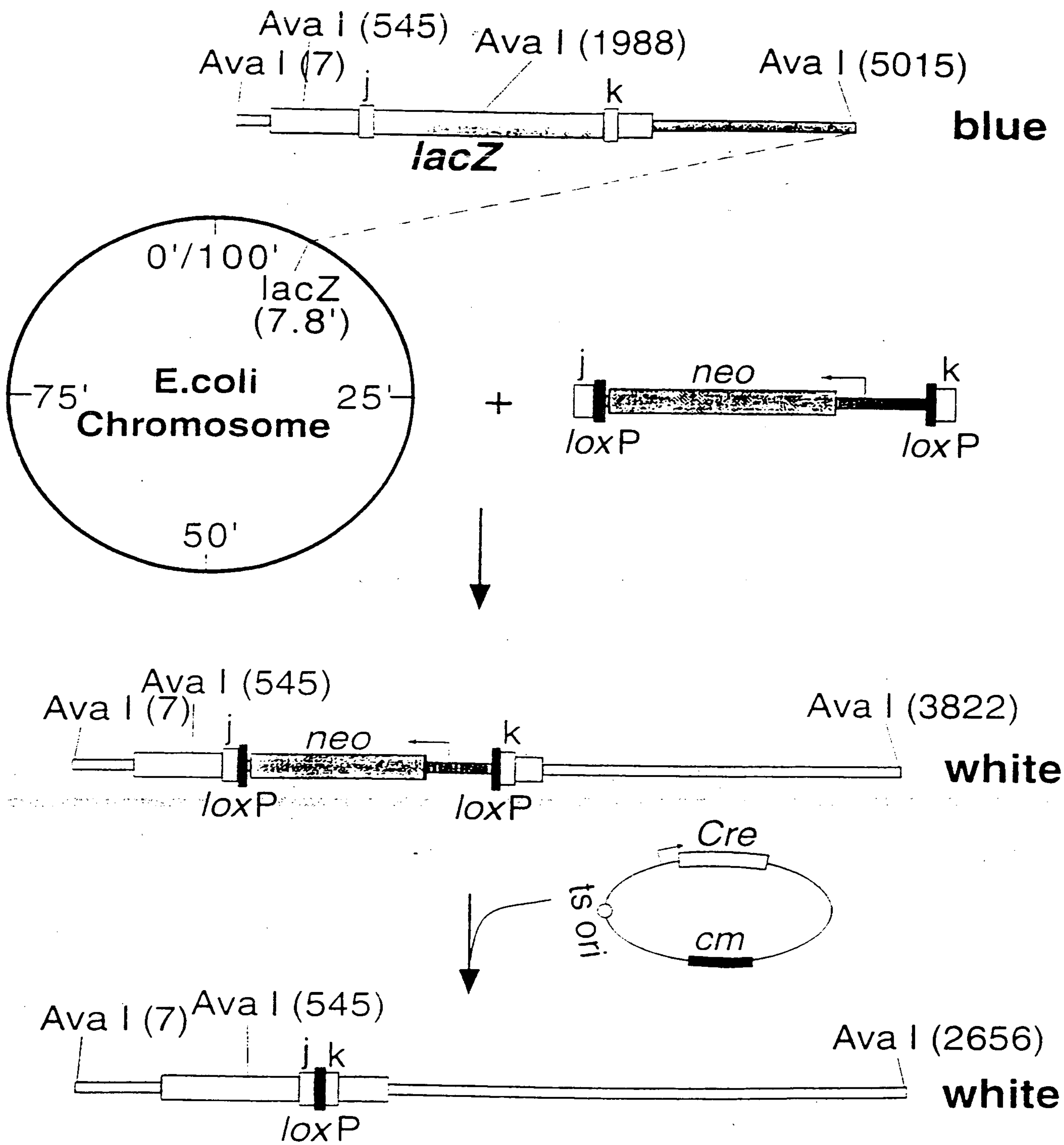
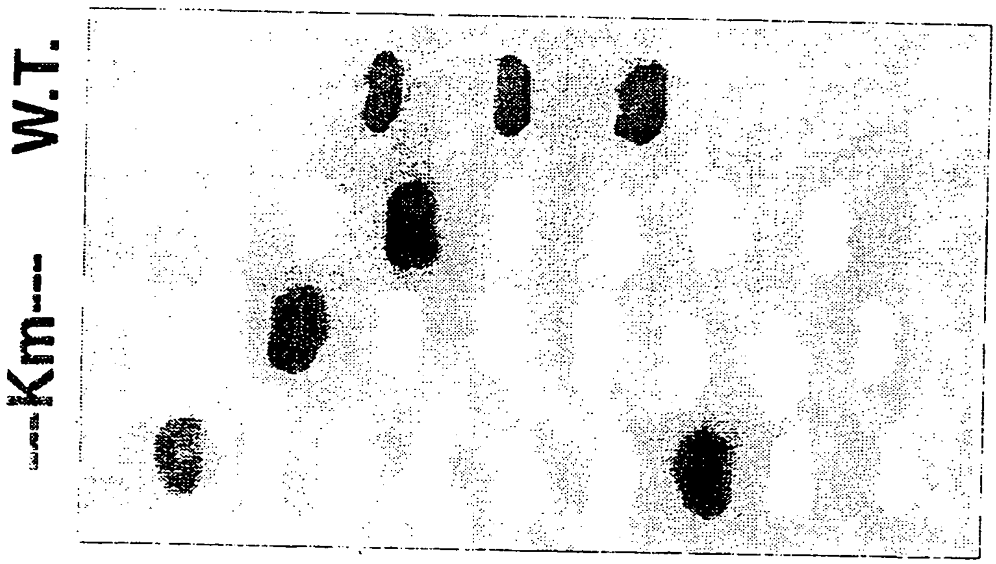

Ava I (545)
Ava I (1988)
Ava I (7)
Ava I (5015)
j
k
lacZ
blue
0'/100'
lacZ
(7.8')
75'
E.coli
Chromosome
25'
50'
+
j
neo
k
loxP
loxP
Ava I (545)
Ava I (7)
Ava I (3822)
j
neo
k
white
loxP
loxP
Cre
ts ori
cm
Ava I (7)
Ava I (545)
Ava I (2656)
j k
white
loxP

Figure 9

b



c

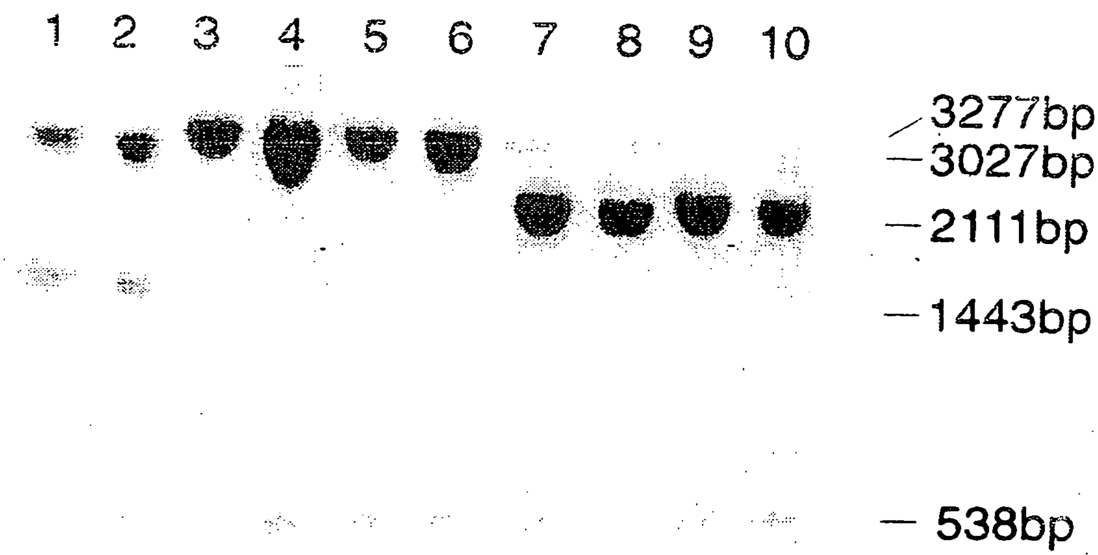


Figure 10a

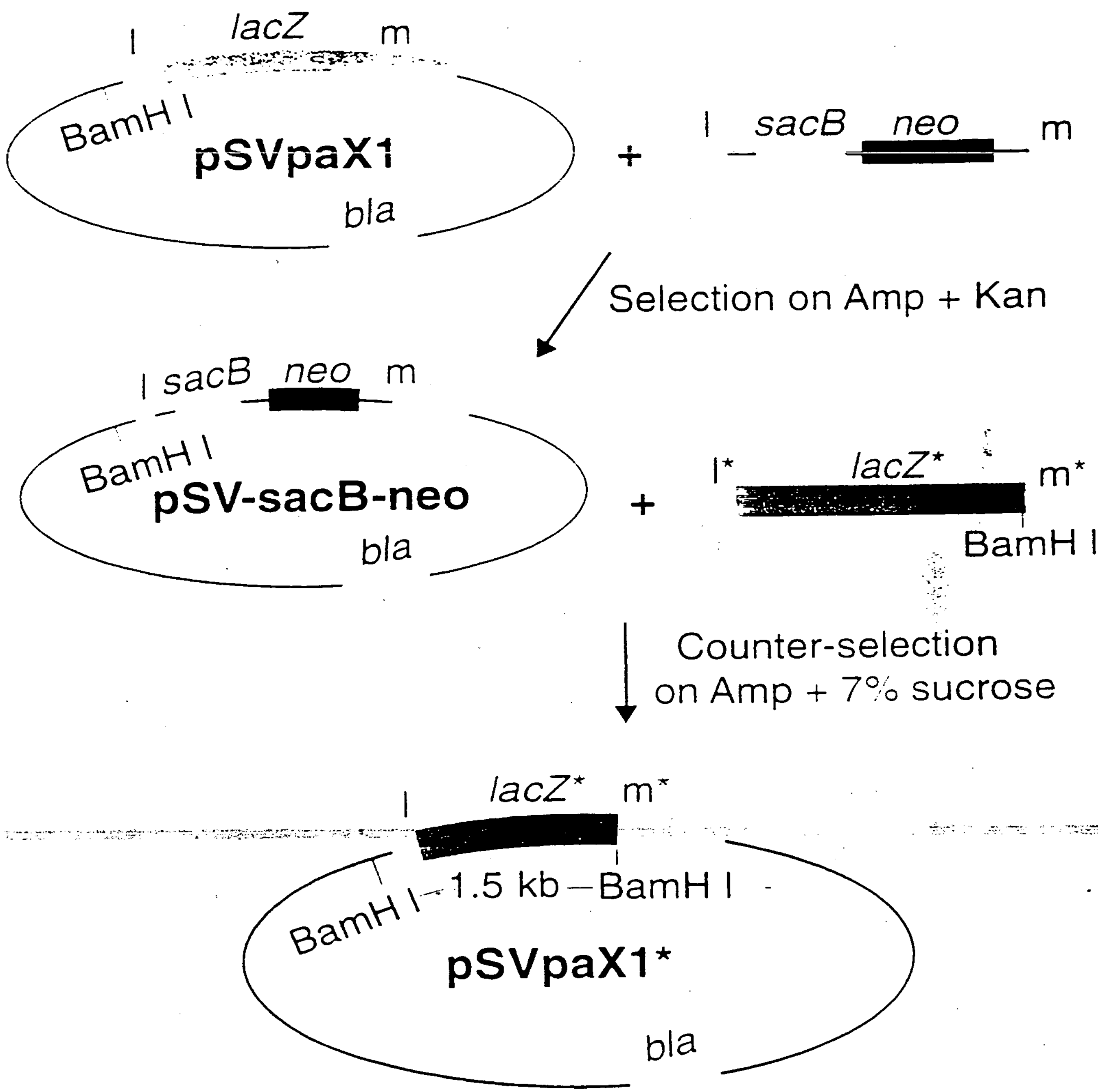
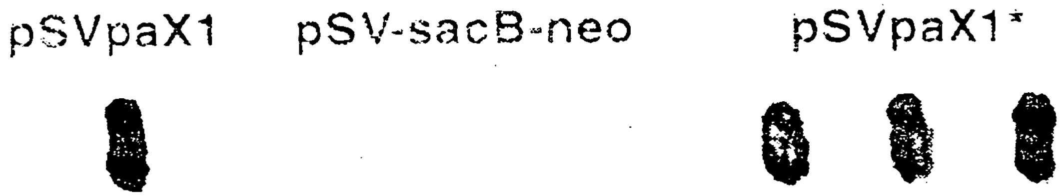

l
lacZ
m
BamH I
pSVpaX1
bla
+
l
sacB
neo
m
Selection on Amp + Kan
l
sacB
neo
m
BamH I
pSV-sacB-neo
bla
+
l*
lacZ*
m*
BamH I
Counter-selection
on Amp + 7% sucrose
l
lacZ*
m*
BamH I
1.5 kb
BamH I
pSVpaX1*
bla

Figure 10

b



c

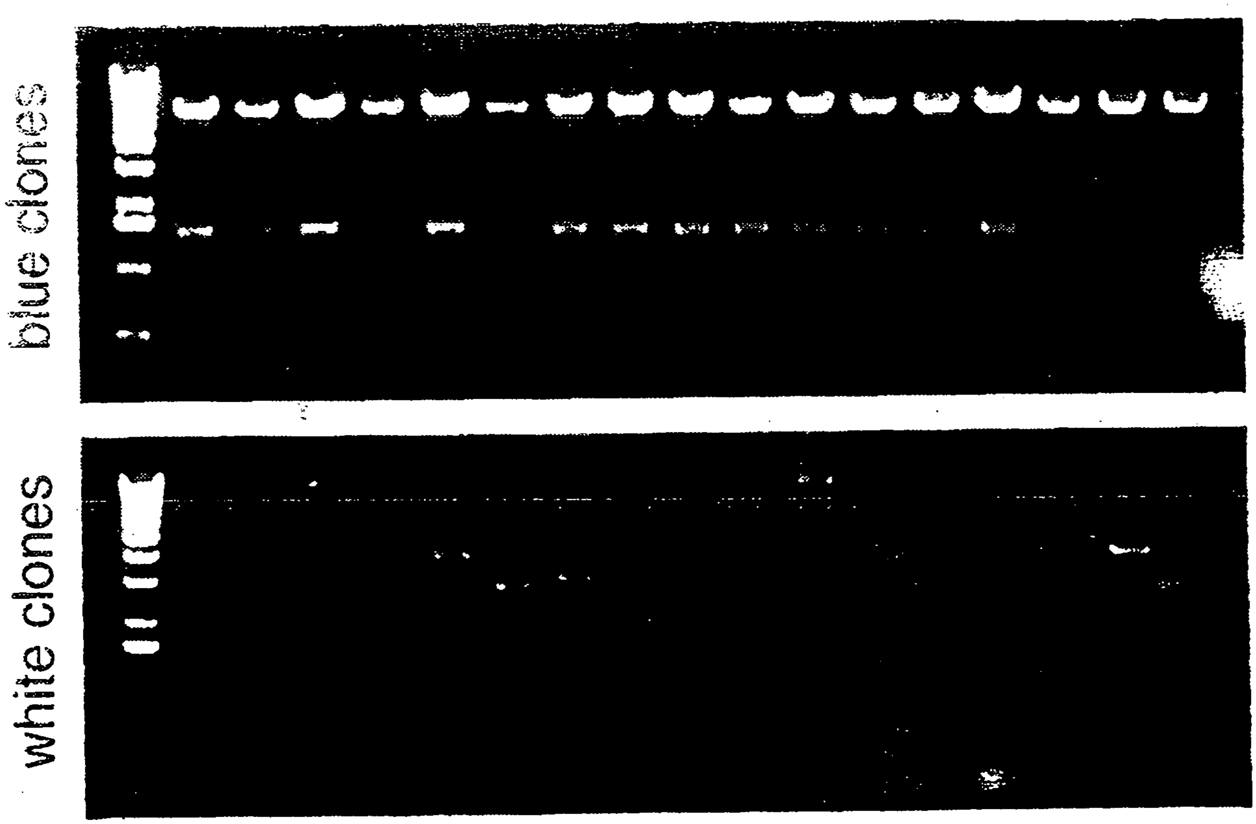


Figure 11a

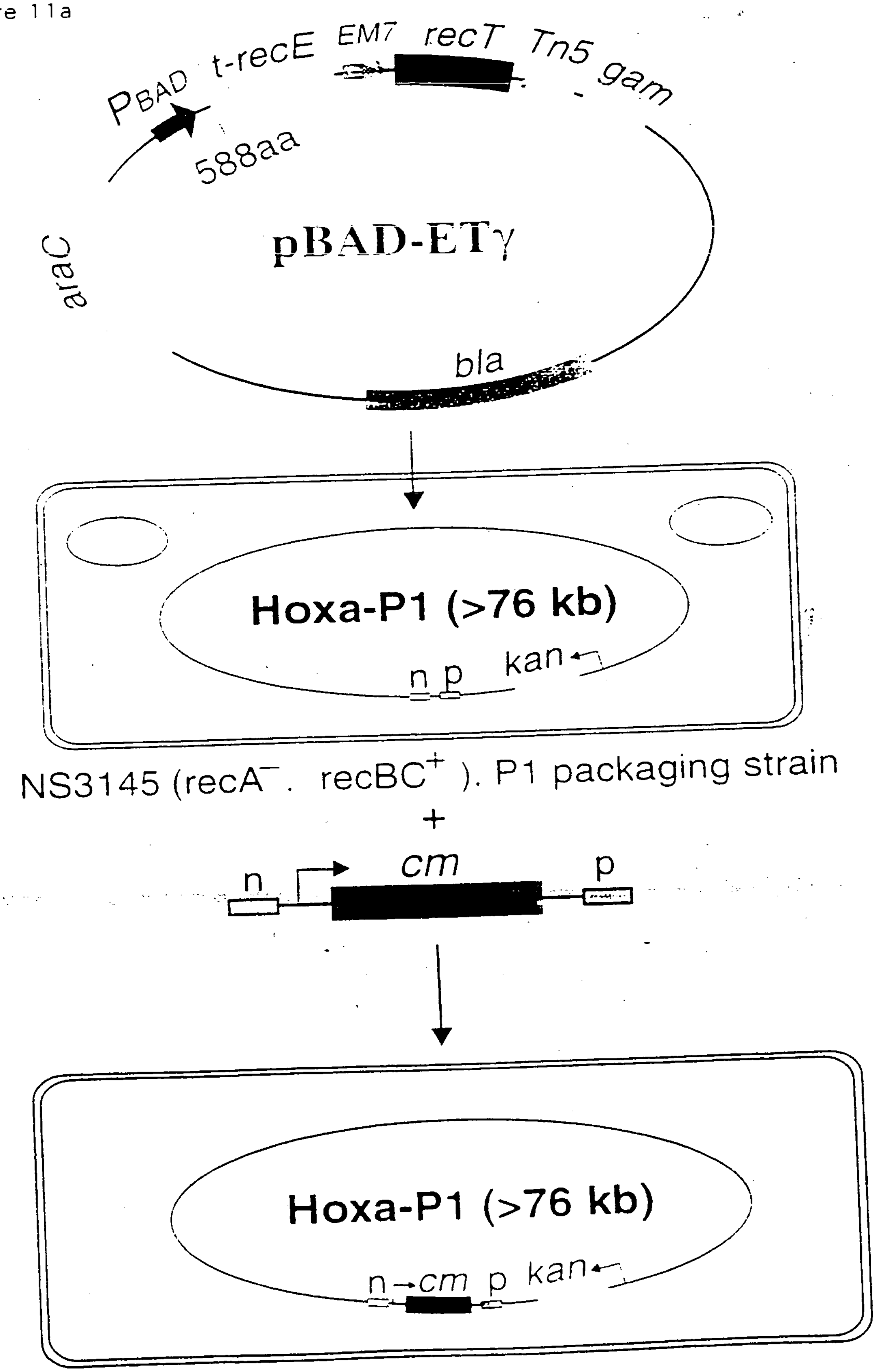

PBAD
t-recE
EM7
recT
Tn5
gam
588aa
pBAD-ETγ
araC
bla
Hoxa-P1 (>76 kb)
n p
kan
NS3145 (recA⁻. recBC⁺). P1 packaging strain
+
n
cm
p
Hoxa-P1 (>76 kb)
n→cm p
kan

Figure 11 b

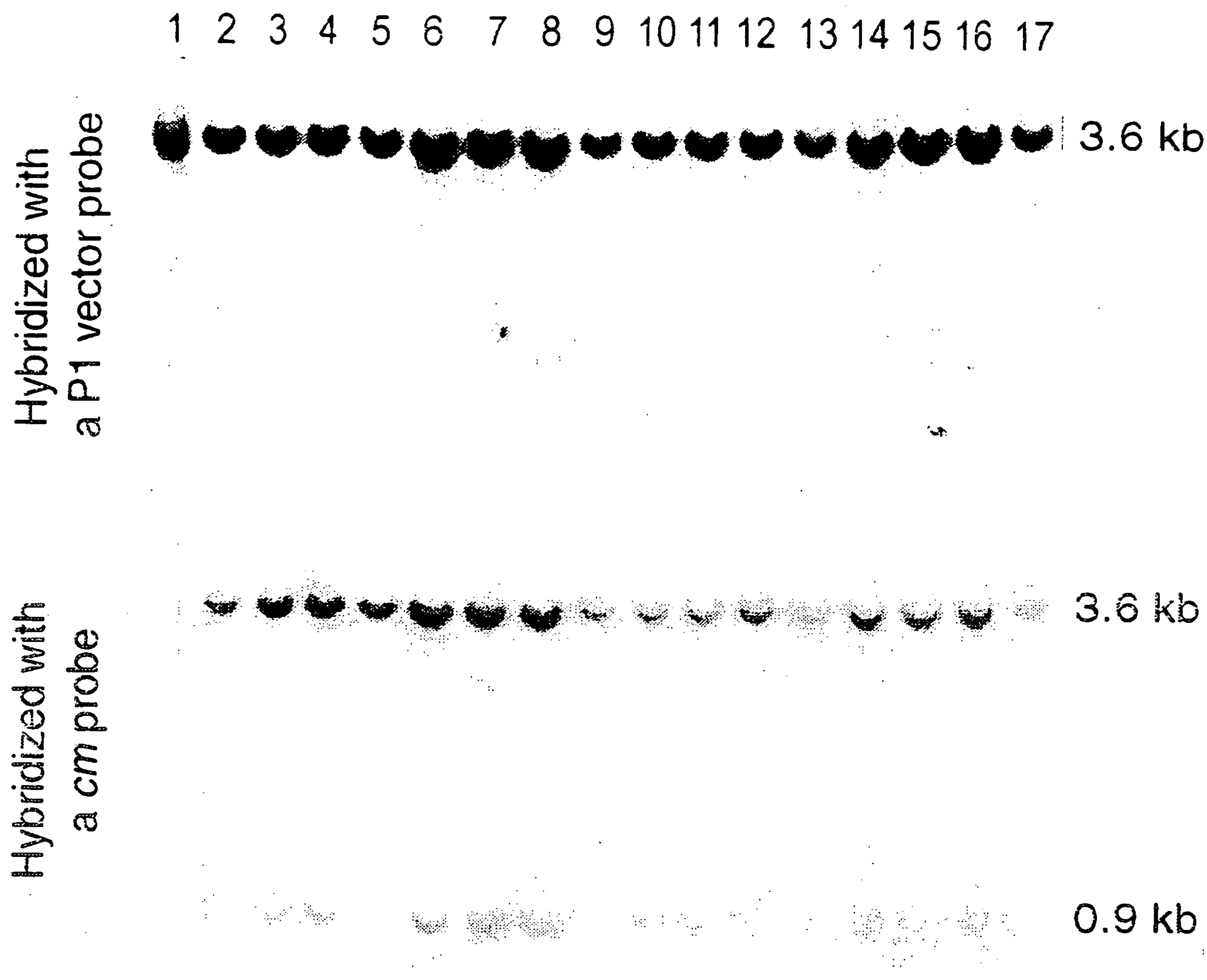
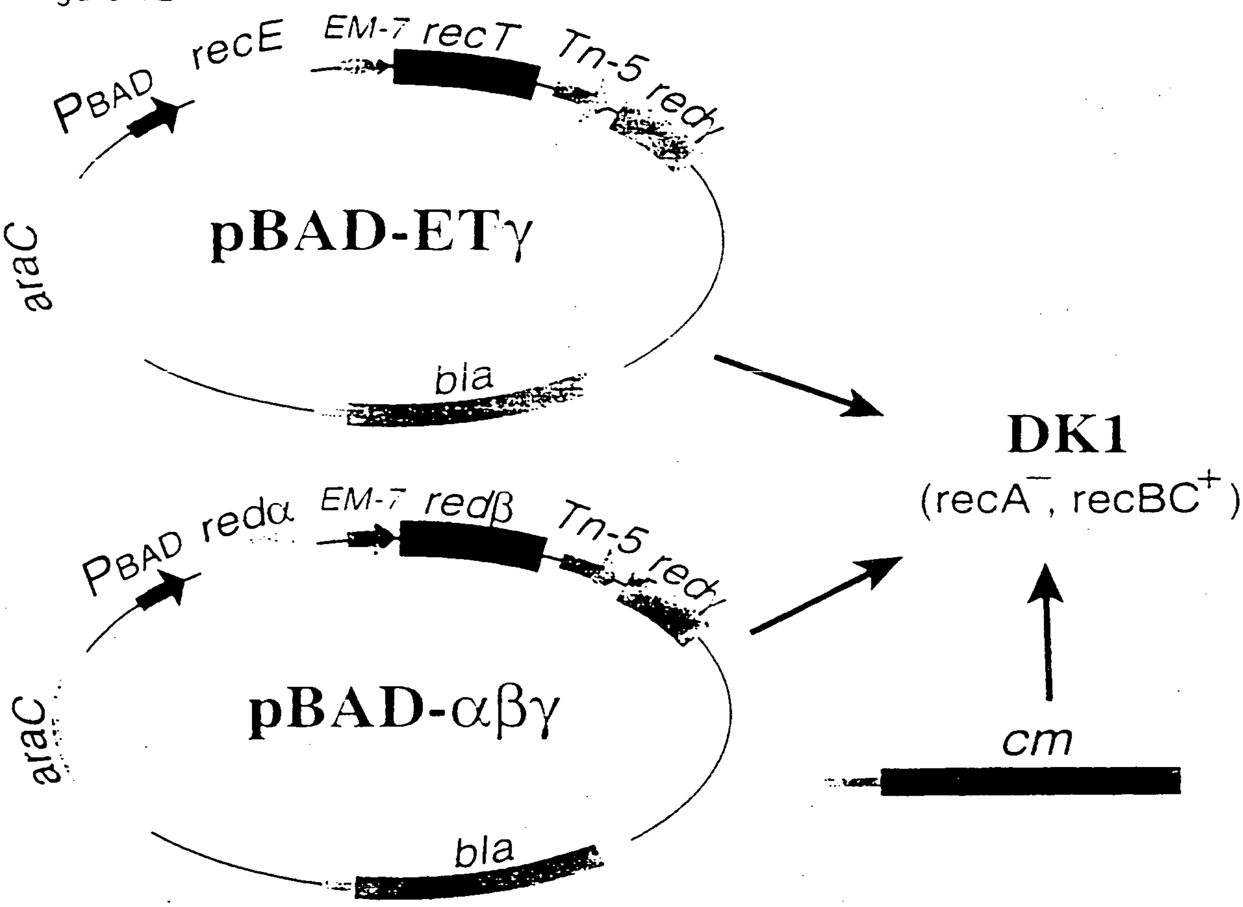

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Hybridized with
a P1 vector probe
3.6 kb
Hybridized with
a *cm* probe
3.6 kb
0.9 kb

Figure 12


recE
PBAD
EM-7 recT
Tn-5 redγ
araC
pBAD-ETγ
bla
DK1
(recA$^-$, recBC$^+$)
PBAD redα
EM-7 redβ
Tn-5 redγ
araC
pBAD-αβγ
bla
cm

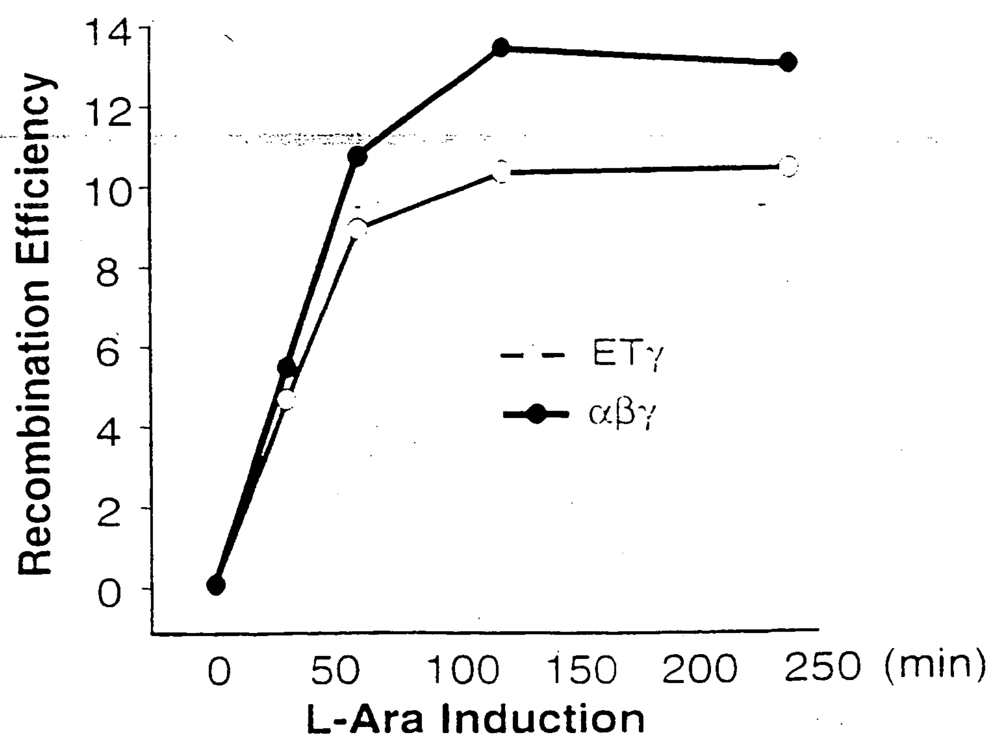

Recombination Efficiency
14
12
10
8
6
4
2
0
0
50
100
150
200
250 (min)
L-Ara Induction
ETγ
αβγ

Figure 13 a

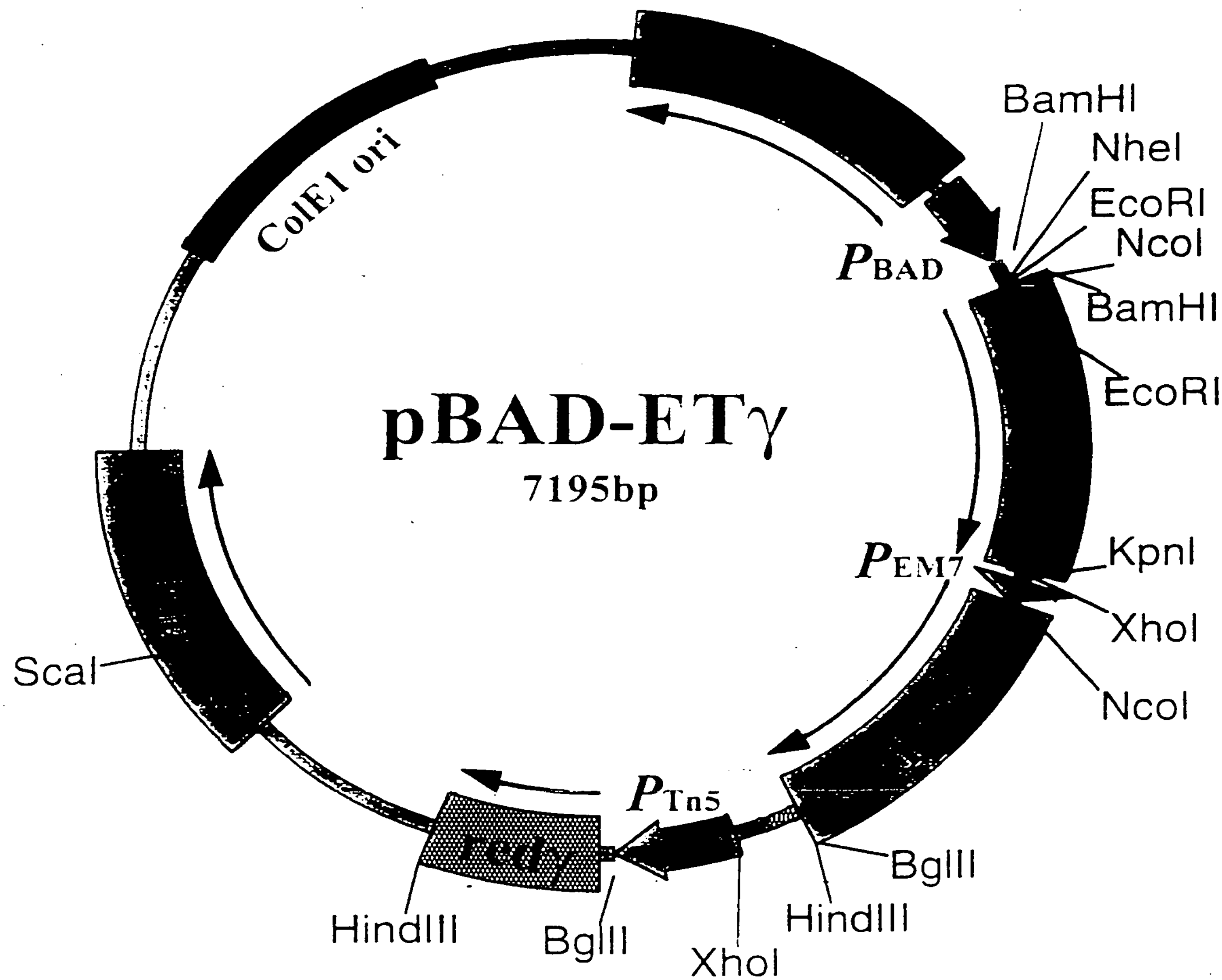

pBAD-ETγ
7195bp
BamHI
NheI
EcoRI
NcoI
BamHI
EcoRI
KpnI
XhoI
NcoI
BglII
HindIII
XhoI
BglII
HindIII
ScaI
ColE1 ori
PBAD
PEM7
PTn5
redγ

Figure 13b

1 ATCGATGCATAATGTGCCTGTCAAATGGACGAAGCAGGG

40 ATTCTGCAAACCCTATGCTACTCCGTCAAGCCGTCAATT

79 GTCTGATTCGTTACCAA TTA TGA CAA CTT GAC
293◀• • • Ser Leu Lys Val

111 GGC TAC ATC ATT CAC TTT TTC TTC ACA ACC
288◀Ala Val Asp Asn Val Lys Glu Glu Cys Gly

141 GGC ACG GAA CTC GCT CGG GCT GGC CCC GGT
278◀Ala Arg Phe Glu Ser Pro Ser Ala Gly Thr

171 GCA TTT TTT AAA TAC CCG CGA GAA ATA GAG
268◀Cys Lys Lys Phe Val Arg Ser Phe Tyr Leu

201 TTG ATC GTC AAA ACC AAC ATT GCG ACC GAC
258◀Gln Asp Asp Phe Gly Val Asn Arg Gly Val

231 GGT GGC GAT AGG CAT CCG GGT GGT GCT CAA
248◀Thr Ala Ile Pro Met Arg Thr Thr Ser Leu

261 AAG CAG CTT CGC CTG GCT GAT ACG TTG GTC
238◀Leu Leu Lys Ala Gln Ser Ile Arg Gln Asp

291 CTC GCG CCA GCT TAA GAC GCT AAT CCC TAA
228◀Glu Arg Trp Ser Leu Val Ser Ile Gly Leu

321 CTG CTG GCG GAA AAG ATG TGA CAG ACG CGA
218◀Gln Gln Arg Phe Leu His Ser Leu Arg Ser

351 CGG CGA CAA GCA AAC ATG CTG TGC GAC GCT
208◀Pro Ser Leu Cys Val His Gln Ala Val Ser

381 GGC GAT ATC AAA ATT GCT GTC TGC CAG GTG
198◀Ala Ile Asp Phe Asn Ser Asp Ala Leu His

411 ATC GCT GAT GTA CTG ACA AGC CTC GCG TAC

Figure 13b (cont'd)

188◀Asp Ser Ile Tyr Gln Cys Ala Glu Arg Val

441 CCG ATT ATC CAT CGG TGG ATG GAG CGA CTC

178◀Arg Asn Asp Met Pro Pro His Leu Ser Glu

471 GTT AAT CGC TTC CAT GCG CCG CAG TAA CAA

168◀Asn Ile Ala Glu Met Arg Arg Leu Leu Leu

501 TTG CTC AAG CAG ATT TAT CGC CAG CAG CTC

158◀Gln Glu Leu Leu Asn Ile Ala Leu Leu Glu

531 CGA ATA GCG CCC TTC CCC TTG CCC GGC GTT

148◀Ser Tyr Arg Gly Glu Gly Gln Gly Ala Asn

561 AAT GAT TTG CCC AAA CAG GTC GCT GAA ATG

138◀Ile Ile Gln Gly Phe Leu Asp Ser Phe His

591 CGG CTG GTG CGC TTC ATC CGG GCG AAA GAA

128◀Pro Gln His Ala Glu Asp Pro Arg Phe Phe

621 CCC CGT ATT GGC AAA TAT TGA CGG CCA GTT

118◀Gly Thr Asn Ala Phe Ile Ser Pro Trp Asn

651 AAG CCA TTC ATG CCA GTA GGC GCG CGG ACG

108◀Leu Trp Glu His Trp Tyr Ala Arg Pro Arg

681 AAA GTA AAC CCA CTG GTG ATA CCA TTC GCG

98◀Phe Tyr Val Trp Gln His Tyr Trp Glu Arg

711 AGC CTC CGG ATG ACG ACC GTA GTG ATG AAT

88◀Ala Glu Pro His Arg Gly Tyr His His Ile

741 CTC TCC TGG CGG GAA CAG CAA AAT ATC ACC

78◀Glu Gly Pro Pro Phe Leu Leu Ile Asp Gly

771 CGG TCG GCA AAC AAA TTC TCG TCC CTG ATT

68◀Pro Arg Cys Val Phe Glu Arg Gly Gln Asn

Figure 13b (cont'd)

801 TTT CAC CAC CCC CTG ACC GCG AAT GGT GAG
58◄Lys Val Val Gly Gln Gly Arg Ile Thr Leu

831 ATT GAG AAT ATA ACC TTT CAT TCC CAG CGG
48◄Asn Leu Ile Tyr Gly Lys Met Gly Leu Pro

861 TCG GTC GAT AAA AAA ATC GAG ATA ACC GTT
38◄Arg Asp Ile Phe Phe Asp Leu Tyr Gly Asn

891 GGC CTC AAT CGG CGT TAA ACC CGC CAC CAG
28◄Ala Glu Ile Pro Thr Leu Gly Ala Val Leu

921 ATG GGC ATT AAA CGA GTA TCC CGG CAG CAG
18◄His Ala Asn Phe Ser Tyr Gly Pro Leu Leu

951 GGG ATC ATT TTG CGC TTC AGC CAT ACTTTTC
8◄Pro Asp Asn Gln Ala Glu Ala Met

982 ATACTCCCGCCATTCAGAGAAGAAACCAATTGTCCATAT

1021 TGCATCAGACATTGCCGTCACTGCGTCTTTTACTGGCTC

1060 TTCTCGCTAACCAAACCGGTAACCCCGCTTATTAAAAGC

1099 ATTCTGTAACAAAGCGGGACCAAAGCCATGACAAAAACG

1138 CGTAACAAAAGTGTCTATAATCACGGCAGAAAAGTCCAC

1177 ATTGATTATTTGCACGGCGTCACACTTTGCTATGCCATA

BamHI

1216 GCATTTTTATCCATAAGATTAGCGGATCCTACCTGACGC

Figure 13b (cont'd)

1255 TTTTTATCGCAACTCTCTACTGTTTCTCCATACCCGTTT

Nhel EcoRI NcoI BamHI

1294 TTTTGGGCTAGCAGGAGGAAT TCACC ATG GAT CCC
1▶Met Asp Pro

1329 GTA ATC GTA GAA GAC ATA GAG CCA GGT ATT
4▶Val Ile Val Glu Asp Ile Glu Pro Gly Ile

1359 TAT TAC GGA ATT TCG AAT GAG AAT TAC CAC
14▶Tyr Tyr Gly Ile Ser Asn Glu Asn Tyr His

1389 GCG GGT CCC GGT ATC AGT AAG TCT CAG CTC
24▶Ala Gly Pro Gly Ile Ser Lys Ser Gln Leu

1419 GAT GAC ATT GCT GAT ACT CCG GCA CTA TAT
34▶Asp Asp Ile Ala Asp Thr Pro Ala Leu Tyr

1449 TTG TGG CGT AAA AAT GCC CCC GTG GAC ACC
44▶Leu Trp Arg Lys Asn Ala Pro Val Asp Thr

1479 ACA AAG ACA AAA ACG CTC GAT TTA GGA ACT
54▶Thr Lys Thr Lys Thr Leu Asp Leu Gly Thr

1509 GCT TTC CAC TGC CGG GTA CTT GAA CCG GAA
64▶Ala Phe His Cys Arg Val Leu Glu Pro Glu

EcoRI

1539 GAA TTC AGT AAC CGC TTT ATC GTA GCA CCT
74▶Glu Phe Ser Asn Arg Phe Ile Val Ala Pro

1569 GAA TTT AAC CGC CGT ACA AAC GCC GGA AAA
84▶Glu Phe Asn Arg Arg Thr Asn Ala Gly Lys

1599 GAA GAA GAG AAA GCG TTT CTG ATG GAA TGC
94▶Glu Glu Glu Lys Ala Phe Leu Met Glu Cys

1629 GCA AGC ACA GGA AAA ACG GTT ATC ACT GCG
104▶Ala Ser Thr Gly Lys Thr Val Ile Thr Ala

Figure 13b (cont'd)

1659 GAA GAA GGC CGG AAA ATT GAA CTC ATG TAT
114▶ Glu Glu Gly Arg Lys Ile Glu Leu Met Tyr

1689 CAA AGC GTT ATG GCT TTG CCG CTG GGG CAA
124▶ Gln Ser Val Met Ala Leu Pro Leu Gly Gln

1719 TGG CTT GTT GAA AGC GCC GGA CAC GCT GAA
134▶ Trp Leu Val Glu Ser Ala Gly His Ala Glu

1749 TCA TCA ATT TAC TGG GAA GAT CCT GAA ACA
144▶ Ser Ser Ile Tyr Trp Glu Asp Pro Glu Thr

1779 GGA ATT TTG TGT CGG TGC CGT CCG GAC AAA
154▶ Gly Ile Leu Cys Arg Cys Arg Pro Asp Lys

1809 ATT ATC CCT GAA TTT CAC TGG ATC ATG GAC
164▶ Ile Ile Pro Glu Phe His Trp Ile Met Asp

1839 GTG AAA ACT ACG GCG GAT ATT CAA CGA TTC
174▶ Val Lys Thr Thr Ala Asp Ile Gln Arg Phe

1869 AAA ACC GCT TAT TAC GAC TAC CGC TAT CAC
184▶ Lys Thr Ala Tyr Tyr Asp Tyr Arg Tyr His

1899 GTT CAG GAT GCA TTC TAC AGT GAC GGT TAT
194▶ Val Gln Asp Ala Phe Tyr Ser Asp Gly Tyr

1929 GAA GCA CAG TTT GGA GTG CAG CCA ACT TTC
204▶ Glu Ala Gln Phe Gly Val Gln Pro Thr Phe

1959 GTT TTT CTG GTT GCC AGC ACA ACT ATT GAA
214▶ Val Phe Leu Val Ala Ser Thr Thr Ile Glu

1989 TGC GGA CGT TAT CCG GTT GAA ATT TTC ATG
224▶ Cys Gly Arg Tyr Pro Val Glu Ile Phe Met

2019 ATG GGC GAA GAA GCA AAA CTG GCA GGT CAA
234▶ Met Gly Glu Glu Ala Lys Leu Ala Gly Gln

Figure 13b (cont'd)

```
2049  CAG GAA TAT CAC CGC AAT CTG CGA ACC CTG
 244▶ Gln Glu Tyr His Arg Asn Leu Arg Thr Leu

2079  TCT GAC TGC CTG AAT ACC GAT GAA TGG CCA
 254▶ Ser Asp Cys Leu Asn Thr Asp Glu Trp Pro

2109  GCT ATT AAG ACA TTA TCA CTG CCC CGC TGG
 264▶ Ala Ile Lys Thr Leu Ser Leu Pro Arg Trp
                                         XhoI   KpnI
2139  GCT AAG GAA TAT GCA AAT GAC TAGATCTCGAG
 274▶ Ala Lys Glu Tyr Ala Asn Asp

2171  GTACCCGAGCACGTGTTGACAATTAATCATCGGCATAGT

2210  ATATCGGCATAGTATAATACGACAAGGTGAGGAACTAAA
      NcoI
2249  CC ATG GCT AAG CAA CCA CCA ATC GCA AAA
        1▶ Met Ala Lys Gln Pro Pro Ile Ala Lys

2278  GCC GAT CTG CAA AAA ACT CAG GGA AAC CGT
  10▶ Ala Asp Leu Gln Lys Thr Gln Gly Asn Arg

2308  GCA CCA GCA GCA GTT AAA AAT AGC GAC GTG
  20▶ Ala Pro Ala Ala Val Lys Asn Ser Asp Val

2338  ATT AGT TTT ATT AAC CAG CCA TCA ATG AAA
  30▶ Ile Ser Phe Ile Asn Gln Pro Ser Met Lys

2368  GAG CAA CTG GCA GCA GCT CTT CCA CGC CAT
  40▶ Glu Gln Leu Ala Ala Ala Leu Pro Arg His

2398  ATG ACG GCT GAA CGT ATG ATC CGT ATC GCC
  50▶ Met Thr Ala Glu Arg Met Ile Arg Ile Ala

2428  ACC ACA GAA ATT CGT AAA GTT CCG GCG TTA
  60▶ Thr Thr Glu Ile Arg Lys Val Pro Ala Leu
```

Figure 13b (cont'd)

2458	GGA	AAC	TGT	GAC	ACT	ATG	AGT	TTT	GTC	AGT
70▶	Gly	Asn	Cys	Asp	Thr	Met	Ser	Phe	Val	Ser
2488	GCG	ATC	GTA	CAG	TGT	TCA	CAG	CTC	GGA	CTT
80▶	Ala	Ile	Val	Gln	Cys	Ser	Gln	Leu	Gly	Leu
2518	GAG	CCA	GGT	AGC	GCC	CTC	GGT	CAT	GCA	TAT
90▶	Glu	Pro	Gly	Ser	Ala	Leu	Gly	His	Ala	Tyr
2548	TTA	CTG	CCT	TTT	GGT	AAT	AAA	AAC	GAA	AAG
100▶	Leu	Leu	Pro	Phe	Gly	Asn	Lys	Asn	Glu	Lys
2578	AGC	GGT	AAA	AAG	AAC	GTT	CAG	CTA	ATC	ATT
110▶	Ser	Gly	Lys	Lys	Asn	Val	Gln	Leu	Ile	Ile
2608	GGC	TAT	CGC	GGC	ATG	ATT	GAT	CTG	GCT	CGC
120▶	Gly	Tyr	Arg	Gly	Met	Ile	Asp	Leu	Ala	Arg
2638	CGT	TCT	GGT	CAA	ATC	GCC	AGC	CTG	TCA	GCC
130▶	Arg	Ser	Gly	Gln	Ile	Ala	Ser	Leu	Ser	Ala
2668	CGT	GTT	GTC	CGT	GAA	GGT	GAC	GAG	TTT	AGC
140▶	Arg	Val	Val	Arg	Glu	Gly	Asp	Glu	Phe	Ser
2698	TTC	GAA	TTT	GGC	CTT	GAT	GAA	AAG	TTA	ATA
150▶	Phe	Glu	Phe	Gly	Leu	Asp	Glu	Lys	Leu	Ile
2728	CAC	CGC	CCG	GGA	GAA	AAC	GAA	GAT	GCC	CCG
160▶	His	Arg	Pro	Gly	Glu	Asn	Glu	Asp	Ala	Pro
2758	GTT	ACC	CAC	GTC	TAT	GCT	GTC	GCA	AGA	CTG
170▶	Val	Thr	His	Val	Tyr	Ala	Val	Ala	Arg	Leu
2788	AAA	GAC	GGA	GGT	ACT	CAG	TTT	GAA	GTT	ATG
180▶	Lys	Asp	Gly	Gly	Thr	Gln	Phe	Glu	Val	Met
2818	ACG	CGC	AAA	CAG	ATT	GAG	CTG	GTG	CGC	AGC
190▶	Thr	Arg	Lys	Gln	Ile	Glu	Leu	Val	Arg	Ser

Figure 13b (cont'd)

2848 CTG AGT AAA GCT GGT AAT AAC GGG CCG TGG
200▶Leu Ser Lys Ala Gly Asn Asn Gly Pro Trp

2878 GTA ACT CAC TGG GAA GAA ATG GCA AAG AAA
210▶Val Thr His Trp Glu Glu Met Ala Lys Lys

2908 ACG GCT ATT CGT CGC CTG TTC AAA TAT TTG
220▶Thr Ala Ile Arg Arg Leu Phe Lys Tyr Leu

2938 CCC GTA TCA ATT GAG ATC CAG CGT GCA GTA
230▶Pro Val Ser Ile Glu Ile Gln Arg Ala Val

2968 TCA ATG GAT GAA AAG GAA CCA CTG ACA ATC
240▶Ser Met Asp Glu Lys Glu Pro Leu Thr Ile

2998 GAT CCT GCA GAT TCC TCT GTA TTA ACC GGG
250▶Asp Pro Ala Asp Ser Ser Val Leu Thr Gly

3028 GAA TAC AGT GTA ATC GAT AAT TCA GAG GAA
260▶Glu Tyr Ser Val Ile Asp Asn Ser Glu Glu

BglII HindIII
3058 TAG ATCTAAGCTTCCTGCTGAACATCAAAGGCAAGAAA
270▶• • •

3096 ACATCTGTTGTCAAAGACAGCATCCTTGAACAAGGACAA

3135 TTAACAGTTAACAAATAAAAACGCAAAAGAAAATGCCGA

3174 TATCCTATTGGCATTTTCTTTTATTTCTTATCAACATAA

XhoI
3213 AGGTGAATCCCATACCTCGAGCTTCACGCTGCCGCAAGC

3252 ACTCAGGGCGCAAGGGCTGCTAAAAGGAAGCGGAACACG

3291 TAGAAAGCCAGTCCGCAGAAACGGTGCTGACCCCGGATG

3330 AATGTCAGCTACTGGGCTATCTGGACAAGGGAAAACGCA

3369 AGCGCAAAGAGAAAGCAGGTAGCTTGCAGTGGGCTTACA

Figure 13b (cont'd)

3408 TGGCGATAGCTAGACTGGGCGGTTTTATGGACAGCAAGC

3447 GAACCGGAATTGCCAGCTGGGGCGCCCTCTGGTAAGGTT

3486 GGGAAGCCCTGCAAAGTAAACTGGATGGCTTTCTTGCCG

BglII

3525 CCAAGGATCTGATGGCGCAGGGGATCAAGATCTGATCAA

3564 GAGACAGGATGAGGATCGTTTCGC ATG GAT ATT
1▶Met Asp Ile

3597 AAT ACT GAA ACT GAG ATC AAG CAA AAG CAT
4▶Asn Thr Glu Thr Glu Ile Lys Gln Lys His

3627 TCA CTA ACC CCC TTT CCT GTT TTC CTA ATC
14▶Ser Leu Thr Pro Phe Pro Val Phe Leu Ile

3657 AGC CCG GCA TTT CGC GGG CGA TAT TTT CAC
24▶Ser Pro Ala Phe Arg Gly Arg Tyr Phe His

3687 AGC TAT TTC AGG AGT TCA GCC ATG AAC GCT
34▶Ser Tyr Phe Arg Ser Ser Ala Met Asn Ala

3717 TAT TAC ATT CAG GAT CGT CTT GAG GCT CAG
44▶Tyr Tyr Ile Gln Asp Arg Leu Glu Ala Gln

3747 AGC TGG GCG CGT CAC TAC CAG CAG CTC GCC
54▶Ser Trp Ala Arg His Tyr Gln Gln Leu Ala

3777 CGT GAA GAG AAA GAG GCA GAA CTG GCA GAC
64▶Arg Glu Glu Lys Glu Ala Glu Leu Ala Asp

3807 GAC ATG GAA AAA GGC CTG CCC CAG CAC CTG
74▶Asp Met Glu Lys Gly Leu Pro Gln His Leu

3837 TTT GAA TCG CTA TGC ATC GAT CAT TTG CAA
84▶Phe Glu Ser Leu Cys Ile Asp His Leu Gln

3867 CGC CAC GGG GCC AGC AAA AAA TCC ATT ACC
94▶Arg His Gly Ala Ser Lys Lys Ser Ile Thr

Figure 13b (cont'd)

3897 CGT GCG TTT GAT GAC GAT GTT GAG TTT CAG
104▶Arg Ala Phe Asp Asp Asp Val Glu Phe Gln

3927 GAG CGC ATG GCA GAA CAC ATC CGG TAC ATG
114▶Glu Arg Met Ala Glu His Ile Arg Tyr Met

3957 GTT GAA ACC ATT GCT CAC CAC CAG GTT GAT
124▶Val Glu Thr Ile Ala His His Gln Val Asp

HindIII
3987 ATT GAT TCA GAG GTA TAA AACGAGTAGA AGCT
134▶Ile Asp Ser Glu Val •••

4019 TGGCTGTTTTGGCGGATGAGAGAAGATTTTCAGCCTGAT

4058 ACAGATTAAATCAGAACGCAGAAGCGGTCTGATAAAACA

4097 GAATTTGCCTGGCGGCAGTAGCGCGGTGGTCCCACCTGA

4136 CCCCATGCCGAACTCAGAAGTGAAACGCCGTAGCGCCGA

4175 TGGTAGTGTGGGGTCTCCCCATGCGAGAGTAGGGAACTG

4214 CCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACT

4253 GGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACGCTC

4292 TCCTGAGTAGGACAAATCCGCCGGGAGCGGATTTGAACG

4331 TTGCGAAGCAACGGCCCGGAGGGTGGCGGGCAGGACGCC

4370 CGCCATAAACTGCCAGGCATCAAATTAAGCAGAAGGCCA

4409 TCCTGACGGATGGCCTTTTTGCGTTTCTACAAACTCTTT

4448 TGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTC

4487 ATGAGACAATAACCCTGATAAATGCTTCAATAATATTGA

4526 AAAAGGAAGAGT ATG AGT ATT CAA CAT TTC
1▶Met Ser Ile Gln His Phe

Figure 13b (cont'd)

4556	CGT	GTC	GCC	CTT	ATT	CCC	TTT	TTT	GCG	GCA
7▶	Arg	Val	Ala	Leu	Ile	Pro	Phe	Phe	Ala	Ala
4586	TTT	TGC	CTT	CCT	GTT	TTT	GCT	CAC	CCA	GAA
17▶	Phe	Cys	Leu	Pro	Val	Phe	Ala	His	Pro	Glu
4616	ACG	CTG	GTG	AAA	GTA	AAA	GAT	GCT	GAA	GAT
27▶	Thr	Leu	Val	Lys	Val	Lys	Asp	Ala	Glu	Asp
4646	CAG	TTG	GGT	GCA	CGA	GTG	GGT	TAC	ATC	GAA
37▶	Gln	Leu	Gly	Ala	Arg	Val	Gly	Tyr	Ile	Glu
4676	CTG	GAT	CTC	AAC	AGC	GGT	AAG	ATC	CTT	GAG
47▶	Leu	Asp	Leu	Asn	Ser	Gly	Lys	Ile	Leu	Glu
4706	AGT	TTT	CGC	CCC	GAA	GAA	CGT	TTT	CCA	ATG
57▶	Ser	Phe	Arg	Pro	Glu	Glu	Arg	Phe	Pro	Met
4736	ATG	AGC	ACT	TTT	AAA	GTT	CTG	CTA	TGT	GGC
67▶	Met	Ser	Thr	Phe	Lys	Val	Leu	Leu	Cys	Gly
4766	GCG	GTA	TTA	TCC	CGT	GTT	GAC	GCC	GGG	CAA
77▶	Ala	Val	Leu	Ser	Arg	Val	Asp	Ala	Gly	Gln
4796	GAG	CAA	CTC	GGT	CGC	CGC	ATA	CAC	TAT	TCT
87▶	Glu	Gln	Leu	Gly	Arg	Arg	Ile	His	Tyr	Ser
							ScaI			
4826	CAG	AAT	GAC	TTG	GTT	GAG	TAC	TCA	CCA	GTC
97▶	Gln	Asn	Asp	Leu	Val	Glu	Tyr	Ser	Pro	Val
4856	ACA	GAA	AAG	CAT	CTT	ACG	GAT	GGC	ATG	ACA
107▶	Thr	Glu	Lys	His	Leu	Thr	Asp	Gly	Met	Thr
4886	GTA	AGA	GAA	TTA	TGC	AGT	GCT	GCC	ATA	ACC
117▶	Val	Arg	Glu	Leu	Cys	Ser	Ala	Ala	Ile	Thr
4916	ATG	AGT	GAT	AAC	ACT	GCG	GCC	AAC	TTA	CTT
127▶	Met	Ser	Asp	Asn	Thr	Ala	Ala	Asn	Leu	Leu

Figure 13b (cont'd)

4946	CTG	ACA	ACG	ATC	GGA	GGA	CCG	AAG	GAG	CTA
137▶	Leu	Thr	Thr	Ile	Gly	Gly	Pro	Lys	Glu	Leu
4976	ACC	GCT	TTT	TTG	CAC	AAC	ATG	GGG	GAT	CAT
147▶	Thr	Ala	Phe	Leu	His	Asn	Met	Gly	Asp	His
5006	GTA	ACT	CGC	CTT	GAT	CGT	TGG	GAA	CCG	GAG
157▶	Val	Thr	Arg	Leu	Asp	Arg	Trp	Glu	Pro	Glu
5036	CTG	AAT	GAA	GCC	ATA	CCA	AAC	GAC	GAG	CGT
167▶	Leu	Asn	Glu	Ala	Ile	Pro	Asn	Asp	Glu	Arg
5066	GAC	ACC	ACG	ATG	CCT	GTA	GCA	ATG	GCA	ACA
177▶	Asp	Thr	Thr	Met	Pro	Val	Ala	Met	Ala	Thr
5096	ACG	TTG	CGC	AAA	CTA	TTA	ACT	GGC	GAA	CTA
187▶	Thr	Leu	Arg	Lys	Leu	Leu	Thr	Gly	Glu	Leu
5126	CTT	ACT	CTA	GCT	TCC	CGG	CAA	CAA	TTA	ATA
197▶	Leu	Thr	Leu	Ala	Ser	Arg	Gln	Gln	Leu	Ile
5156	GAC	TGG	ATG	GAG	GCG	GAT	AAA	GTT	GCA	GGA
207▶	Asp	Trp	Met	Glu	Ala	Asp	Lys	Val	Ala	Gly
5186	CCA	CTT	CTG	CGC	TCG	GCC	CTT	CCG	GCT	GGC
217▶	Pro	Leu	Leu	Arg	Ser	Ala	Leu	Pro	Ala	Gly
5216	TGG	TTT	ATT	GCT	GAT	AAA	TCT	GGA	GCC	GGT
227▶	Trp	Phe	Ile	Ala	Asp	Lys	Ser	Gly	Ala	Gly
5246	GAG	CGT	GGG	TCT	CGC	GGT	ATC	ATT	GCA	GCA
237▶	Glu	Arg	Gly	Ser	Arg	Gly	Ile	Ile	Ala	Ala
5276	CTG	GGG	CCA	GAT	GGT	AAG	CCC	TCC	CGT	ATC
247▶	Leu	Gly	Pro	Asp	Gly	Lys	Pro	Ser	Arg	Ile
5306	GTA	GTT	ATC	TAC	ACG	ACG	GGG	AGT	CAG	GCA
257▶	Val	Val	Ile	Tyr	Thr	Thr	Gly	Ser	Gln	Ala

Figure 13b (cont'd)

5336 ACT ATG GAT GAA CGA AAT AGA CAG ATC GCT
267▶ Thr Met Asp Glu Arg Asn Arg Gln Ile Ala

5366 GAG ATA GGT GCC TCA CTG ATT AAG CAT TGG
277▶ Glu Ile Gly Ala Ser Leu Ile Lys His Trp

5396 TAA CTGTCAGACCAAGTTTACTCATATATACTTTAGAT
287▶ • • •

5434 TGATTTACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGG

5473 GTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCA

5512 GCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCT

5551 TTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAA

5590 ATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTAC

5629 GGCACCTCGACCCCAAAAAACTTGATTTGGGTGATGGTT

5668 CACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCC

5707 CTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCT

5746 TGTTCCAAACTTGAACAACACTCAACCCTATCTCGGGCT

5785 ATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGCCT

5824 ATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACG

5863 CGAATTTTAACAAAATATTAACGTTTACAATTTAAAAGG

5902 ATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAA

5941 ATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGAC

5980 CCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTT

6019 TTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCA

6058 CCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTA

6097 CCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCG

Figure 13b (cont'd)

6136 CAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTA
6175 GGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATAC
6214 CTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGT
6253 GGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGA
6292 TAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGG
6331 GGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTAC
6370 ACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGC
6409 GCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCG
6448 GTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAG
6487 CTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTC
6526 GGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGA
6565 TGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGC
6604 AACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCT
6643 TTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCT
6682 GTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACC
6721 GCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTG
6760 AGCGAGGAAGCGGAAGAGCGCCTGATGCGGTATTTTCTC
6799 CTTACGCATCTGTGCGGTATTTCACACCGCATAGGGTCA
6838 TGGCTGCGCCCCGACACCCGCCAACACCCGCTGACGCGC
6877 CCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGAC
6916 AAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGT
6955 TTTCACCGTCATCACCGAAACGCGCGAGGCAGCAAGGAG

Figure 13b (cont'd)

6994 ATGGCGCCCAACAGTCCCCCGGCCACGGGGCCTGCCACC
7033 ATACCCACGCCGAAACAAGCGCTCATGAGCCCGAAGTGG
7072 CGAGCCCGATCTTCCCCATCGGTGATGTCGGCGATATAG
7111 GCGCCAGCAACCGCACCTGTGGCGCCGGTGATGCCGGCC
7150 ACGATGCGTCCGGCGTAGAGGATCTGCTCATGTTTGACA
7189 GCTTATC

Figure 14 a

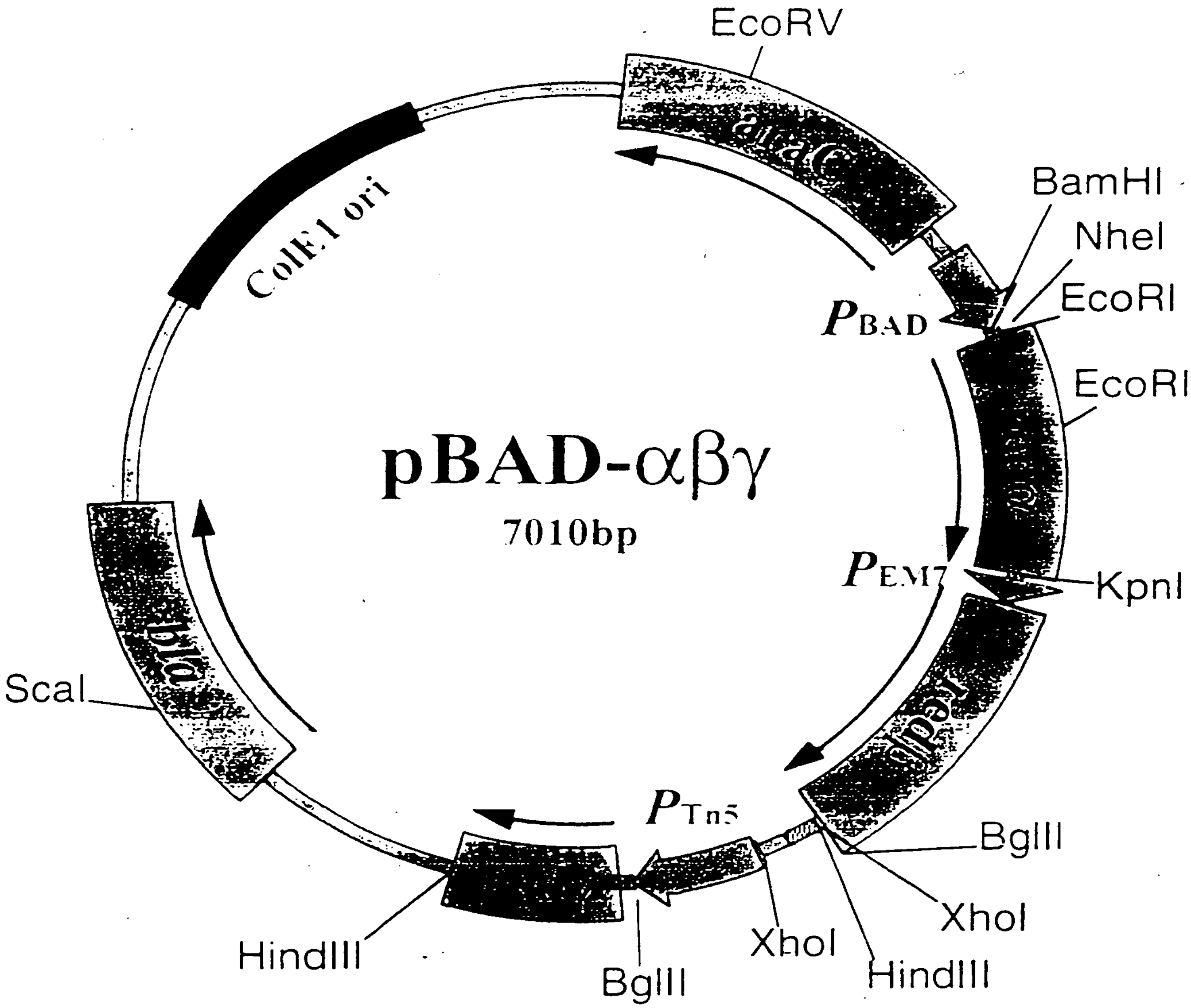

EcoRV
araC
BamHI
NheI
EcoRI
EcoRI
P_{BAD}
ColE1 ori
pBAD-αβγ
7010bp
P_{EM7}
KpnI
bla
ScaI
P_{Tn5}
BglII
XhoI
XhoI
HindIII
HindIII
BglII

Figure 14b

NsiI

1 ATCGATGCATAATGTGCCTGTCAAATGGACGAAGCAGGG

40 ATTCTGCAAACCCTATGCTACTCCGTCAAGCCGTCAATT

79 GTCTGATTCGTTACCAA TTA TGA CAA CTT GAC
293◀• • • Ser Leu Lys Val

111 GGC TAC ATC ATT CAC TTT TTC TTC ACA ACC
288◀Ala Val Asp Asn Val Lys Glu Glu Cys Gly

141 GGC ACG GAA CTC GCT CGG GCT GGC CCC GGT
278◀Ala Arg Phe Glu Ser Pro Ser Ala Gly Thr

171 GCA TTT TTT AAA TAC CCG CGA GAA ATA GAG
268◀Cys Lys Lys Phe Val Arg Ser Phe Tyr Leu

201 TTG ATC GTC AAA ACC AAC ATT GCG ACC GAC
258◀Gln Asp Asp Phe Gly Val Asn Arg Gly Val

231 GGT GGC GAT AGG CAT CCG GGT GGT GCT CAA
248◀Thr Ala Ile Pro Met Arg Thr Thr Ser Leu

261 AAG CAG CTT CGC CTG GCT GAT ACG TTG GTC
238◀Leu Leu Lys Ala Gln Ser Ile Arg Gln Asp

291 CTC GCG CCA GCT TAA GAC GCT AAT CCC TAA
228◀Glu Arg Trp Ser Leu Val Ser Ile Gly Leu

321 CTG CTG GCG GAA AAG ATG TGA CAG ACG CGA
218◀Gln Gln Arg Phe Leu His Ser Leu Arg Ser

351 CGG CGA CAA GCA AAC ATG CTG TGC GAC GCT
208◀Pro Ser Leu Cys Val His Gln Ala Val Ser

EcoRV

381 GGC GAT ATC AAA ATT GCT GTC TGC CAG GTG
198◀Ala Ile Asp Phe Asn Ser Asp Ala Leu His

Figure 14b (cont'd)

411	ATC	GCT	GAT	GTA	CTG	ACA	AGC	CTC	GCG	TAC
188◀	Asp	Ser	Ile	Tyr	Gln	Cys	Ala	Glu	Arg	Val
441	CCG	ATT	ATC	CAT	CGG	TGG	ATG	GAG	CGA	CTC
178◀	Arg	Asn	Asp	Met	Pro	Pro	His	Leu	Ser	Glu
471	GTT	AAT	CGC	TTC	CAT	GCG	CCG	CAG	TAA	CAA
168◀	Asn	Ile	Ala	Glu	Met	Arg	Arg	Leu	Leu	Leu
501	TTG	CTC	AAG	CAG	ATT	TAT	CGC	CAG	CAG	CTC
158◀	Gln	Glu	Leu	Leu	Asn	Ile	Ala	Leu	Leu	Glu
531	CGA	ATA	GCG	CCC	TTC	CCC	TTG	CCC	GGC	GTT
148◀	Ser	Tyr	Arg	Gly	Glu	Gly	Gln	Gly	Ala	Asn
561	AAT	GAT	TTG	CCC	AAA	CAG	GTC	GCT	GAA	ATG
138◀	Ile	Ile	Gln	Gly	Phe	Leu	Asp	Ser	Phe	His
591	CGG	CTG	GTG	CGC	TTC	ATC	CGG	GCG	AAA	GAA
128◀	Pro	Gln	His	Ala	Glu	Asp	Pro	Arg	Phe	Phe
621	CCC	CGT	ATT	GGC	AAA	TAT	TGA	CGG	CCA	GTT
118◀	Gly	Thr	Asn	Ala	Phe	Ile	Ser	Pro	Trp	Asn
651	AAG	CCA	TTC	ATG	CCA	GTA	GGC	GCG	CGG	ACG
108◀	Leu	Trp	Glu	His	Trp	Tyr	Ala	Arg	Pro	Arg
681	AAA	GTA	AAC	CCA	CTG	GTG	ATA	CCA	TTC	GCG
98◀	Phe	Tyr	Val	Trp	Gln	His	Tyr	Trp	Glu	Arg
711	AGC	CTC	CGG	ATG	ACG	ACC	GTA	GTG	ATG	AAT
88◀	Ala	Glu	Pro	His	Arg	Gly	Tyr	His	His	Ile
741	CTC	TCC	TGG	CGG	GAA	CAG	CAA	AAT	ATC	ACC
78◀	Glu	Gly	Pro	Pro	Phe	Leu	Leu	Ile	Asp	Gly
771	CGG	TCG	GCA	AAC	AAA	TTC	TCG	TCC	CTG	ATT
68◀	Pro	Arg	Cys	Val	Phe	Glu	Arg	Gly	Gln	Asn

Figure 14b (cont'd)

801 TTT CAC CAC CCC CTG ACC GCG AAT GGT GAG
58◀Lys Val Val Gly Gln Gly Arg Ile Thr Leu

831 ATT GAG AAT ATA ACC TTT CAT TCC CAG CGG
48◀Asn Leu Ile Tyr Gly Lys Met Gly Leu Pro

861 TCG GTC GAT AAA AAA ATC GAG ATA ACC GTT
38◀Arg Asp Ile Phe Phe Asp Leu Tyr Gly Asn

891 GGC CTC AAT CGG CGT TAA ACC CGC CAC CAG
28◀Ala Glu Ile Pro Thr Leu Gly Ala Val Leu

921 ATG GGC ATT AAA CGA GTA TCC CGG CAG CAG
18◀His Ala Asn Phe Ser Tyr Gly Pro Leu Leu

951 GGG ATC ATT TTG CGC TTC AGC CAT ACTTTTC
8◀Pro Asp Asn Gln Ala Glu Ala Met

982 ATACTCCCGCCATTCAGAGAAGAAACCAATTGTCCATAT

1021 TGCATCAGACATTGCCGTCACTGCGTCTTTTACTGGCTC

1060 TTCTCGCTAACCAAACCGGTAACCCCGCTTATTAAAAGC

1099 ATTCTGTAACAAAGCGGGACCAAAGCCATGACAAAAACG

1138 CGTAACAAAAGTGTCTATAATCACGGCAGAAAAGTCCAC

1177 ATTGATTATTTGCACGGCGTCACACTTTGCTATGCCATA

BamHI
1216 GCATTTTTATCCATAAGATTAGCGGATCCTACCTGACGC

1255 TTTTTATCGCAACTCTCTACTGTTTCTCCATACCCGTTT

NheI EcoRI
1294 TTTTGGCTAGCAGGAGGAATTCACC ATG ACA CCG
1▶Met Thr Pro

PstI
1329 GAC ATT ATC CTG CAG CGT ACC GGG ATC GAT

Figure 14b (cont'd)

4▸	Asp	Ile	Ile	Leu	Gln	Arg	Thr	Gly	Ile	Asp
1359	GTG	AGA	GCT	GTC	GAA	CAG	GGG	GAT	GAT	GCG
14▸	Val	Arg	Ala	Val	Glu	Gln	Gly	Asp	Asp	Ala
1389	TGG	CAC	AAA	TTA	CGG	CTC	GGC	GTC	ATC	ACC
24▸	Trp	His	Lys	Leu	Arg	Leu	Gly	Val	Ile	Thr
1419	GCT	TCA	GAA	GTT	CAC	AAC	GTG	ATA	GCA	AAA
34▸	Ala	Ser	Glu	Val	His	Asn	Val	Ile	Ala	Lys
1449	CCC	CGC	TCC	GGA	AAG	AAG	TGG	CCT	GAC	ATG
44▸	Pro	Arg	Ser	Gly	Lys	Lys	Trp	Pro	Asp	Met
1479	AAA	ATG	TCC	TAC	TTC	CAC	ACC	CTG	CTT	GCT
54▸	Lys	Met	Ser	Tyr	Phe	His	Thr	Leu	Leu	Ala
1509	GAG	GTT	TGC	ACC	GGT	GTG	GCT	CCG	GAA	GTT
64▸	Glu	Val	Cys	Thr	Gly	Val	Ala	Pro	Glu	Val
1539	AAC	GCT	AAA	GCA	CTG	GCC	TGG	GGA	AAA	CAG
74▸	Asn	Ala	Lys	Ala	Leu	Ala	Trp	Gly	Lys	Gln
										EcoRI
1569	TAC	GAG	AAC	GAC	GCC	AGA	ACC	CTG	TTT	GAA
84▸	Tyr	Glu	Asn	Asp	Ala	Arg	Thr	Leu	Phe	Glu
1599	TTC	ACT	TCC	GGC	GTG	AAT	GTT	ACT	GAA	TCC
94▸	Phe	Thr	Ser	Gly	Val	Asn	Val	Thr	Glu	Ser
1629	CCG	ATC	ATC	TAT	CGC	GAC	GAA	AGT	ATG	CGT
104▸	Pro	Ile	Ile	Tyr	Arg	Asp	Glu	Ser	Met	Arg
1659	ACC	GCC	TGC	TCT	CCC	GAT	GGT	TTA	TGC	AGT
114▸	Thr	Ala	Cys	Ser	Pro	Asp	Gly	Leu	Cys	Ser
1689	GAC	GGC	AAC	GGC	CTT	GAA	CTG	AAA	TGC	CCG
124▸	Asp	Gly	Asn	Gly	Leu	Glu	Leu	Lys	Cys	Pro

Figure 14b (cont'd)

1719 TTT ACC TCC CGG GAT TTC ATG AAG TTC CGG
134▶ Phe Thr Ser Arg Asp Phe Met Lys Phe Arg

1749 CTC GGT GGT TTC GAG GCC ATA AAG TCA GCT
144▶ Leu Gly Gly Phe Glu Ala Ile Lys Ser Ala

1779 TAC ATG GCC CAG GTG CAG TAC AGC ATG TGG
154▶ Tyr Met Ala Gln Val Gln Tyr Ser Met Trp

1809 GTG ACG CGA AAA AAT GCC TGG TAC TTT GCC
164▶ Val Thr Arg Lys Asn Ala Trp Tyr Phe Ala

1839 AAC TAT GAC CCG CGT ATG AAG CGT GAA GGC
174▶ Asn Tyr Asp Pro Arg Met Lys Arg Glu Gly

1869 CTG CAT TAT GTC GTG ATT GAG CGG GAT GAA
184▶ Leu His Tyr Val Val Ile Glu Arg Asp Glu

1899 AAG TAC ATG GCG AGT TTT GAC GAG ATC GTG
194▶ Lys Tyr Met Ala Ser Phe Asp Glu Ile Val

1929 CCG GAG TTC ATC GAA AAA ATG GAC GAG GCA
204▶ Pro Glu Phe Ile Glu Lys Met Asp Glu Ala

1959 CTG GCT GAA ATT GGT TTT GTA TTT GGG GAG
214▶ Leu Ala Glu Ile Gly Phe Val Phe Gly Glu

KpnI

1989 CAA TGG CGA TAGATCCGGTACCCGAGCACGTGTTGA
224▶ Gln Trp Arg • • •

2025 CAATTAATCATCGGCATAGTATATCGGCATAGTATAATA

2064 CGACAAGGTGAGGAACTAAACC ATG AGT ACT GCA
1▶ Met Ser Thr Ala

2098 CTC GCA ACG CTG GCT GGG AAG CTG GCT GAA
5▶ Leu Ala Thr Leu Ala Gly Lys Leu Ala Glu

Figure 14b (cont'd)

SalI

2128 CGT GTC GGC ATG GAT TCT GTC GAC CCA CAG
15▶ Arg Val Gly Met Asp Ser Val Asp Pro Gln

2158 GAA CTG ATC ACC ACT CTT CGC CAG ACG GCA
25▶ Glu Leu Ile Thr Thr Leu Arg Gln Thr Ala

2188 TTT AAA GGT GAT GCC AGC GAT GCG CAG TTC
35▶ Phe Lys Gly Asp Ala Ser Asp Ala Gln Phe

2218 ATC GCA TTA CTG ATC GTT GCC AAC CAG TAC
45▶ Ile Ala Leu Leu Ile Val Ala Asn Gln Tyr

2248 GGC CTT AAT CCG TGG ACG AAA GAA ATT TAC
55▶ Gly Leu Asn Pro Trp Thr Lys Glu Ile Tyr

2278 GCC TTT CCT GAT AAG CAG AAT GGC ATC GTT
65▶ Ala Phe Pro Asp Lys Gln Asn Gly Ile Val

2308 CCG GTG GTG GGC GTT GAT GGC TGG TCC CGC
75▶ Pro Val Val Gly Val Asp Gly Trp Ser Arg

2338 ATC ATC AAT GAA AAC CAG CAG TTT GAT GGC
85▶ Ile Ile Asn Glu Asn Gln Gln Phe Asp Gly

2368 ATG GAC TTT GAG CAG GAC AAT GAA TCC TGT
95▶ Met Asp Phe Glu Gln Asp Asn Glu Ser Cys

2398 ACA TGC CGG ATT TAC CGC AAG GAC CGT AAT
105▶ Thr Cys Arg Ile Tyr Arg Lys Asp Arg Asn

2428 CAT CCG ATC TGC GTT ACC GAA TGG ATG GAT
115▶ His Pro Ile Cys Val Thr Glu Trp Met Asp

2458 GAA TGC CGC CGC GAA CCA TTC AAA ACT CGC
125▶ Glu Cys Arg Arg Glu Pro Phe Lys Thr Arg

2488 GAA GGC AGA GAA ATC ACG GGG CCG TGG CAG
135▶ Glu Gly Arg Glu Ile Thr Gly Pro Trp Gln

Figure 14b (cont'd)

2518	TCG	CAT	CCC	AAA	CGG	ATG	TTA	CGT	CAT	AAA
145▶	Ser	His	Pro	Lys	Arg	Met	Leu	Arg	His	Lys
2548	GCC	ATG	ATT	CAG	TGT	GCC	CGT	CTG	GCC	TTC
155▶	Ala	Met	Ile	Gln	Cys	Ala	Arg	Leu	Ala	Phe
2578	GGA	TTT	GCT	GGT	ATC	TAT	GAC	AAG	GAT	GAA
165▶	Gly	Phe	Ala	Gly	Ile	Tyr	Asp	Lys	Asp	Glu
2608	GCC	GAG	CGC	ATT	GTC	GAA	AAT	ACT	GCA	TAC
175▶	Ala	Glu	Arg	Ile	Val	Glu	Asn	Thr	Ala	Tyr
	PstI									
2638	ACT	GCA	GAA	CGT	CAG	CCG	GAA	CGC	GAC	ATC
185▶	Thr	Ala	Glu	Arg	Gln	Pro	Glu	Arg	Asp	Ile
2668	ACT	CCG	GTT	AAC	GAT	GAA	ACC	ATG	CAG	GAG
195▶	Thr	Pro	Val	Asn	Asp	Glu	Thr	Met	Gln	Glu
2698	ATT	AAC	ACT	CTG	CTG	ATC	GCC	CTG	GAT	AAA
205▶	Ile	Asn	Thr	Leu	Leu	Ile	Ala	Leu	Asp	Lys
2728	ACA	TGG	GAT	GAC	GAC	TTA	TTG	CCG	CTC	TGT
215▶	Thr	Trp	Asp	Asp	Asp	Leu	Leu	Pro	Leu	Cys
2758	TCC	CAG	ATA	TTT	CGC	CGC	GAC	ATT	CGT	GCA
225▶	Ser	Gln	Ile	Phe	Arg	Arg	Asp	Ile	Arg	Ala
2788	TCG	TCA	GAA	CTG	ACA	CAG	GCC	GAA	GCA	GTA
235▶	Ser	Ser	Glu	Leu	Thr	Gln	Ala	Glu	Ala	Val
2818	AAA	GCT	CTT	GGA	TTC	CTG	AAA	CAG	AAA	GCC
245▶	Lys	Ala	Leu	Gly	Phe	Leu	Lys	Gln	Lys	Ala
								BglII XhoI		
2848	GCA	GAG	CAG	AAG	GTG	GCA	GCA	TAGATCTCGAG		
255▶	Ala	Glu	Gln	Lys	Val	Ala	Ala	• • •		

Figure 14b (cont'd)

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     HindIII
2880 AAGCTTCCTGCTGAACATCAAAGGCAAGAAAACATCTGT
2919 TGTCAAAGACAGCATCCTTGAACAAGGACAATTAACAGT
2958 TAACAAATAAAAACGCAAAAGAAAATGCCGATATCCTAT
2997 TGGCATTTTCTTTTATTTCTTATCAACATAAAGGTGAAT
              XhoI
3036 CCCATACCTCGAGCTTCACGCTGCCGCAAGCACTCAGGG
3075 CGCAAGGGCTGCTAAAAGGAAGCGGAACACGTAGAAAGC
3114 CAGTCCGCAGAAACGGTGCTGACCCCGGATGAATGTCAG
3153 CTACTGGGCTATCTGGACAAGGGAAAACGCAAGCGCAAA
3192 GAGAAAGCAGGTAGCTTGCAGTGGGCTTACATGGCGATA
3231 GCTAGACTGGGCGGTTTTATGGACAGCAAGCGAACCGGA
         PvuII
3270 ATTGCCAGCTGGGGCGCCCTCTGGTAAGGTTGGGAAGCC
3309 CTGCAAAGTAAACTGGATGGCTTTCTTGCCGCCAAGGAT
                            BglII
3348 CTGATGGCGCAGGGGATCAAGATCTGATCAAGAGACAGG
3387 ATGAGGATCGTTTCGC ATG GAT ATT AAT ACT
                   1▶Met Asp Ile Asn Thr
3418 GAA ACT GAG ATC AAG CAA AAG CAT TCA CTA
   6▶Glu Thr Glu Ile Lys Gln Lys His Ser Leu
3448 ACC CCC TTT CCT GTT TTC CTA ATC AGC CCG
  16▶Thr Pro Phe Pro Val Phe Leu Ile Ser Pro
3478 GCA TTT CGC GGG CGA TAT TTT CAC AGC TAT
  26▶Ala Phe Arg Gly Arg Tyr Phe His Ser Tyr
3508 TTC AGG AGT TCA GCC ATG AAC GCT TAT TAC
  36▶Phe Arg Ser Ser Ala Met Asn Ala Tyr Tyr
```

Figure 14b (cont'd)

3538	ATT	CAG	GAT	CGT	CTT	GAG	GCT	CAG	AGC	TGG
46▶	Ile	Gln	Asp	Arg	Leu	Glu	Ala	Gln	Ser	Trp
3568	GCG	CGT	CAC	TAC	CAG	CAG	CTC	GCC	CGT	GAA
56▶	Ala	Arg	His	Tyr	Gln	Gln	Leu	Ala	Arg	Glu
3598	GAG	AAA	GAG	GCA	GAA	CTG	GCA	GAC	GAC	ATG
66▶	Glu	Lys	Glu	Ala	Glu	Leu	Ala	Asp	Asp	Met
3628	GAA	AAA	GGC	CTG	CCC	CAG	CAC	CTG	TTT	GAA
76▶	Glu	Lys	Gly	Leu	Pro	Gln	His	Leu	Phe	Glu
3658	TCG	CTA	TGC	ATC	GAT	CAT	TTG	CAA	CGC	CAC
86▶	Ser	Leu	Cys	Ile	Asp	His	Leu	Gln	Arg	His
3688	GGG	GCC	AGC	AAA	AAA	TCC	ATT	ACC	CGT	GCG
96▶	Gly	Ala	Ser	Lys	Lys	Ser	Ile	Thr	Arg	Ala
3718	TTT	GAT	GAC	GAT	GTT	GAG	TTT	CAG	GAG	CGC
106▶	Phe	Asp	Asp	Asp	Val	Glu	Phe	Gln	Glu	Arg
3748	ATG	GCA	GAA	CAC	ATC	CGG	TAC	ATG	GTT	GAA
116▶	Met	Ala	Glu	His	Ile	Arg	Tyr	Met	Val	Glu
3778	ACC	ATT	GCT	CAC	CAC	CAG	GTT	GAT	ATT	GAT
126▶	Thr	Ile	Ala	His	His	Gln	Val	Asp	Ile	Asp

HindIII

3808 TCA GAG GTA TAA AACGAGTAGA AGC TTG GCT

136▶ Ser Glu Val •••

3839 GTT TTG GCG GAT GAG AGA AGA TTT TCA GCC

3869 TGA TACAGATTAAATCAGAACGCAGAAGCGGTCTGATA

3907 AAACAGAATTTGCCTGGCGGCAGTAGCGCGGTGGTCCCA

3946 CCTGACCCCATGCCGAACTCAGAAGTGAAACGCCGTAGC

3985 GCCGATGGTAGTGTGGGGTCTCCCCATGCGAGAGTAGGG

Figure 14b (cont'd)

4024 AACTGCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAA

4063 AGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAA

4102 CGCTCTCCTGAGTAGGACAAATCCGCCGGGAGCGGATTT

4141 GAACGTTGCGAAGCAACGGCCCGGAGGGTGGCGGGCAGG

4180 ACGCCCGCCATAAACTGCCAGGCATCAAATTAAGCAGAA

4219 GGCCATCCTGACGGATGGCCTTTTTGCGTTTCTACAAAC

4258 TCTTTTGTTTATTTTTCTAAATACATTCAAATATGTATC

4297 CGCTCATGAGACAATAACCCTGATAAATGCTTCAATAAT

4336 ATTGAAAAAGGAAGAGT ATG AGT ATT CAA CAT
1▶Met Ser Ile Gln His

4368 TTC CGT GTC GCC CTT ATT CCC TTT TTT GCG
6▶Phe Arg Val Ala Leu Ile Pro Phe Phe Ala

4398 GCA TTT TGC CTT CCT GTT TTT GCT CAC CCA
16▶Ala Phe Cys Leu Pro Val Phe Ala His Pro

4428 GAA ACG CTG GTG AAA GTA AAA GAT GCT GAA
26▶Glu Thr Leu Val Lys Val Lys Asp Ala Glu

4458 GAT CAG TTG GGT GCA CGA GTG GGT TAC ATC
36▶Asp Gln Leu Gly Ala Arg Val Gly Tyr Ile

4488 GAA CTG GAT CTC AAC AGC GGT AAG ATC CTT
46▶Glu Leu Asp Leu Asn Ser Gly Lys Ile Leu

4518 GAG AGT TTT CGC CCC GAA GAA CGT TTT CCA
56▶Glu Ser Phe Arg Pro Glu Glu Arg Phe Pro

4548 ATG ATG AGC ACT TTT AAA GTT CTG CTA TGT
66▶Met Met Ser Thr Phe Lys Val Leu Leu Cys

Figure 14b (cont'd)

4578 GGC GCG GTA TTA TCC CGT GTT GAC GCC GGG
76▶ Gly Ala Val Leu Ser Arg Val Asp Ala Gly

4608 CAA GAG CAA CTC GGT CGC CGC ATA CAC TAT
86▶ Gln Glu Gln Leu Gly Arg Arg Ile His Tyr

ScaI
4638 TCT CAG AAT GAC TTG GTT GAG TAC TCA CCA
96▶ Ser Gln Asn Asp Leu Val Glu Tyr Ser Pro

4668 GTC ACA GAA AAG CAT CTT ACG GAT GGC ATG
106▶ Val Thr Glu Lys His Leu Thr Asp Gly Met

4698 ACA GTA AGA GAA TTA TGC AGT GCT GCC ATA
116▶ Thr Val Arg Glu Leu Cys Ser Ala Ala Ile

4728 ACC ATG AGT GAT AAC ACT GCG GCC AAC TTA
126▶ Thr Met Ser Asp Asn Thr Ala Ala Asn Leu

4758 CTT CTG ACA ACG ATC GGA GGA CCG AAG GAG
136▶ Leu Leu Thr Thr Ile Gly Gly Pro Lys Glu

4788 CTA ACC GCT TTT TTG CAC AAC ATG GGG GAT
146▶ Leu Thr Ala Phe Leu His Asn Met Gly Asp

4818 CAT GTA ACT CGC CTT GAT CGT TGG GAA CCG
156▶ His Val Thr Arg Leu Asp Arg Trp Glu Pro

4848 GAG CTG AAT GAA GCC ATA CCA AAC GAC GAG
166▶ Glu Leu Asn Glu Ala Ile Pro Asn Asp Glu

4878 CGT GAC ACC ACG ATG CCT GTA GCA ATG GCA
176▶ Arg Asp Thr Thr Met Pro Val Ala Met Ala

4908 ACA ACG TTG CGC AAA CTA TTA ACT GGC GAA
186▶ Thr Thr Leu Arg Lys Leu Leu Thr Gly Glu

4938 CTA CTT ACT CTA GCT TCC CGG CAA CAA TTA
196▶ Leu Leu Thr Leu Ala Ser Arg Gln Gln Leu

Figure 14b (cont'd)

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4968  ATA GAC TGG ATG GAG GCG GAT AAA GTT GCA
 206▶ Ile Asp Trp Met Glu Ala Asp Lys Val Ala

4998  GGA CCA CTT CTG CGC TCG GCC CTT CCG GCT
 216▶ Gly Pro Leu Leu Arg Ser Ala Leu Pro Ala

5028  GGC TGG TTT ATT GCT GAT AAA TCT GGA GCC
 226▶ Gly Trp Phe Ile Ala Asp Lys Ser Gly Ala

5058  GGT GAG CGT GGG TCT CGC GGT ATC ATT GCA
 236▶ Gly Glu Arg Gly Ser Arg Gly Ile Ile Ala

5088  GCA CTG GGG CCA GAT GGT AAG CCC TCC CGT
 246▶ Ala Leu Gly Pro Asp Gly Lys Pro Ser Arg

5118  ATC GTA GTT ATC TAC ACG ACG GGG AGT CAG
 256▶ Ile Val Val Ile Tyr Thr Thr Gly Ser Gln

5148  GCA ACT ATG GAT GAA CGA AAT AGA CAG ATC
 266▶ Ala Thr Met Asp Glu Arg Asn Arg Gln Ile

5178  GCT GAG ATA GGT GCC TCA CTG ATT AAG CAT
 276▶ Ala Glu Ile Gly Ala Ser Leu Ile Lys His

5208  TGG TAA CTGTCAGACCAAGTTTACTCATATATACTTT
 286▶ Trp •••

5245  AGATTGATTTACGCGCCCTGTAGCGGCGCATTAAGCGC

5284  GCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTT

5323  GCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCT

5362  TCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCT

5401  CTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCT

5440  TTACGGCACCTCGACCCCAAAAAACTTGATTTGGGTGAT

5479  GGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTT
```

Figure 14b (cont'd)

5518 CGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGA
5557 CTCTTGTTCCAAACTTGAACAACACTCAACCCTATCTCG
5596 GGCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCG
5635 GCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTT
5674 AACGCGAATTTTAACAAAATATTAACGTTTACAATTTAA
5713 AAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGAC
5752 CAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTC
5791 AGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCC
5830 TTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAA
5869 ACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGA
5908 GCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAG
5947 AGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTA
5986 GTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTAC
6025 ATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGC
6064 CAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAG
6103 ACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAAC
6142 GGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGAC
6181 CTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGA
6220 AAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTA
6259 TCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAG
6298 GGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCC
6337 TGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTT

Figure 14b (cont'd)

6376 GTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGC
6415 CAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTG
6454 GCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGA
6493 TTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGA
6532 TACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTC
6571 AGTGAGCGAGGAAGCGGAAGAGCGCCTGATGCGGTATTT
6610 TCTCCTTACGCATCTGTGCGGTATTTCACACCGCATAGG
6649 GTCATGGCTGCGCCCCGACACCCGCCAACACCCGCTGAC
6688 GCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTAC
6727 AGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAG
6766 AGGTTTTCACCGTCATCACCGAAACGCGCGAGGCAGCAA
6805 GGAGATGGCGCCCAACAGTCCCCCGGCCACGGGGCCTGC
6844 CACCATACCCACGCCGAAACAAGCGCTCATGAGCCCGAA
6883 GTGGCGAGCCCGATCTTCCCCATCGGTGATGTCGGCGAT
6922 ATAGGCGCCAGCAACCGCACCTGTGGCGCCGGTGATGCC
6961 GGCCACGATGCGTCCGGCGTAGAGGATCTGCTCATGTTT
7000 GACAGCTTATC

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
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(B) STREET: Meyerhofstrasse 1
(C) CITY: Heidelberg
(E) COUNTRY: DE
(F) POSTAL CODE (ZIP): D-69117

(ii) TITLE OF INVENTION: Novel DNA Cloning Method

(iii) NUMBER OF SEQUENCES: 14

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: EP 97121562.2
(B) FILING DATE: 05-DEC-1997

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: EP 98118756.0
(B) FILING DATE: 05-OCT-1998

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6150 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(vii) IMMEDIATE SOURCE:
(B) CLONE: pBAD24-recET

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:complement (96..974)
(D) OTHER INFORMATION:/product= "araC"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:1320..2162
(D) OTHER INFORMATION:/product= "t-recE"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:2155..2972
(D) OTHER INFORMATION:/product= "recT"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:3493..4353
(D) OTHER INFORMATION:/product= "bla"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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ATCGATGCAT AATGTGCCTG TCAAATGGAC GAAGCAGGGA TTCTGCAAAC CCTATGCTAC     60
TCCGTCAAGC CGTCAATTGT CTGATTCGTT ACCAATTATG ACAACTTGAC GGCTACATCA    120
TTCACTTTTT CTTCACAACC GGCACGGAAC TCGCTCGGGC TGGCCCCGGT GCATTTTTTA    180
AATACCCGCG AGAAATAGAG TTGATCGTCA AAACCAACAT TGCGACCGAC GGTGGCGATA    240
GGCATCCGGG TGGTGCTCAA AAGCAGCTTC GCCTGGCTGA TACGTTGGTC CTCGCGCCAG    300
CTTAAGACGC TAATCCCTAA CTGCTGGCGG AAAAGATGTG ACAGACGCGA CGGCGACAAG    360
CAAACATGCT GTGCGACGCT GGCGATATCA AAATTGCTGT CTGCCAGGTG ATCGCTGATG    420
TACTGACAAG CCTCGCGTAC CCGATTATCC ATCGGTGGAT GGAGCGACTC GTTAATCGCT    480
TCCATGCGCC GCAGTAACAA TTGCTCAAGC AGATTTATCG CCAGCAGCTC CGAATAGCGC    540
CCTTCCCCTT GCCCGGCGTT AATGATTTGC CCAAACAGGT CGCTGAAATG CGGCTGGTGC    600
GCTTCATCCG GGCGAAAGAA CCCCGTATTG GCAAATATTG ACGGCCAGTT AAGCCATTCA    660
TGCCAGTAGG CGCGCGGACG AAAGTAAACC CACTGGTGAT ACCATTCGCG AGCCTCCGGA    720
TGACGACCGT AGTGATGAAT CTCTCCTGGC GGGAACAGCA AAATATCACC CGGTCGGCAA    780
ACAAATTCTC GTCCCTGATT TTTCACCACC CCCTGACCGC GAATGGTGAG ATTGAGAATA    840
TAACCTTTCA TTCCCAGCGG TCGGTCGATA AAAAAATCGA GATAACCGTT GGCCTCAATC    900
GGCGTTAAAC CCGCCACCAG ATGGGCATTA AACGAGTATC CCGGCAGCAG GGGATCATTT    960
TGCGCTTCAG CCATACTTTT CATACTCCCG CCATTCAGAG AAGAAACCAA TTGTCCATAT   1020
TGCATCAGAC ATTGCCGTCA CTGCGTCTTT TACTGGCTCT TCTCGCTAAC CAAACCGGTA   1080
ACCCCGCTTA TTAAAAGCAT TCTGTAACAA AGCGGGACCA AAGCCATGAC AAAAACGCGT   1140
AACAAAAGTG TCTATAATCA CGGCAGAAAA GTCCACATTG ATTATTTGCA CGGCGTCACA   1200
CTTTGCTATG CCATAGCATT TTTATCCATA AGATTAGCGG ATCCTACCTG ACGCTTTTTA   1260
TCGCAACTCT CTACTGTTTC TCCATACCCG TTTTTTTGGG CTAGCAGGAG GAATTCACCA   1320
TGGATCCCGT AATCGTAGAA GACATAGAGC CAGGTATTTA TTACGGAATT TCGAATGAGA   1380
ATTACCACGC GGGTCCCGGT ATCAGTAAGT CTCAGCTCGA TGACATTGCT GATACTCCGG   1440
CACTATATTT GTGGCGTAAA AATGCCCCCG TGGACACCAC AAAGACAAAA ACGCTCGATT   1500
TAGGAACTGC TTTCCACTGC CGGGTACTTG AACCGGAAGA ATTCAGTAAC CGCTTTATCG   1560
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TAGCACCTGA ATTTAACCGC CGTACAAACG CCGGAAAAGA AGAAGAGAAA GCGTTTCTGA 1620
TGGAATGCGC AAGCACAGGA AAAACGGTTA TCACTGCGGA AGAAGGCCGG AAAATTGAAC 1680
TCATGTATCA AAGCGTTATG GCTTTGCCGC TGGGGCAATG GCTTGTTGAA AGCGCCGGAC 1740
ACGCTGAATC ATCAATTTAC TGGGAAGATC CTGAAACAGG AATTTTGTGT CGGTGCCGTC 1800
CGGACAAAAT TATCCCTGAA TTTCACTGGA TCATGGACGT GAAAACTACG GCGGATATTC 1860
AACGATTCAA AACCGCTTAT TACGACTACC GCTATCACGT TCAGGATGCA TTCTACAGTG 1920
ACGGTTATGA AGCACAGTTT GGAGTGCAGC CAACTTTCGT TTTTCTGGTT GCCAGCACAA 1980
CTATTGAATG CGGACGTTAT CCGGTTGAAA TTTTCATGAT GGGCGAAGAA GCAAAACTGG 2040
CAGGTCAACA GGAATATCAC CGCAATCTGC GAACCCTGTC TGACTGCCTG AATACCGATG 2100
AATGGCCAGC TATTAAGACA TTATCACTGC CCCGCTGGGC TAAGGAATAT GCAAATGACT 2160
AAGCAACCAC CAATCGCAAA AGCCGATCTG CAAAAAACTC AGGGAAACCG TGCACCAGCA 2220
GCAGTTAAAA ATAGCGACGT GATTAGTTTT ATTAACCAGC CATCAATGAA AGAGCAACTG 2280
GCAGCAGCTC TTCCACGCCA TATGACGGCT GAACGTATGA TCCGTATCGC CACCACAGAA 2340
ATTCGTAAAG TTCCGGCGTT AGGAAACTGT GACACTATGA GTTTTGTCAG TGCGATCGTA 2400
CAGTGTTCAC AGCTCGGACT TGAGCCAGGT AGCGCCCTCG GTCATGCATA TTTACTGCCT 2460
TTTGGTAATA AAAACGAAAA GAGCGGTAAA AAGAACGTTC AGCTAATCAT TGGCTATCGC 2520
GGCATGATTG ATCTGGCTCG CCGTTCTGGT CAAATCGCCA GCCTGTCAGC CCGTGTTGTC 2580
CGTGAAGGTG ACGAGTTTAG CTTCGAATTT GGCCTTGATG AAAAGTTAAT ACACCGCCCG 2640
GGAGAAAACG AAGATGCCCC GGTTACCCAC GTCTATGCTG TCGCAAGACT GAAAGACGGA 2700
GGTACTCAGT TTGAAGTTAT GACGCGCAAA CAGATTGAGC TGGTGCGCAG CCTGAGTAAA 2760
GCTGGTAATA ACGGGCCGTG GGTAACTCAC TGGGAAGAAA TGGCAAAGAA AACGGCTATT 2820
CGTCGCCTGT TCAAATATTT GCCCGTATCA ATTGAGATCC AGCGTGCAGT ATCAATGGAT 2880
GAAAAGGAAC CACTGACAAT CGATCCTGCA GATTCCTCTG TATTAACCGG GGAATACAGT 2940
GTAATCGATA ATTCAGAGGA ATAGATCTAA GCTTGGCTGT TTTGGCGGAT GAGAGAAGAT 3000
TTTCAGCCTG ATACAGATTA AATCAGAACG CAGAAGCGGT CTGATAAAAC AGAATTTGCC 3060
TGGCGGCAGT AGCGCGGTGG TCCCACCTGA CCCCATGCCG AACTCAGAAG TGAAACGCCG 3120
TAGCGCCGAT GGTAGTGTGG GGTCTCCCCA TGCGAGAGTA GGGAACTGCC AGGCATCAAA 3180
TAAAACGAAA GGCTCAGTCG AAAGACTGGG CCTTTCGTTT TATCTGTTGT TTGTCGGTGA 3240
ACGCTCTCCT GAGTAGGACA AATCCGCCGG GAGCGGATTT GAACGTTGCG AAGCAACGGC 3300
CCGGAGGGTG GCGGGCAGGA CGCCCGCCAT AAACTGCCAG GCATCAAATT AAGCAGAAGG 3360
CCATCCTGAC GGATGGCCTT TTTGCGTTTC TACAAACTCT TTTGTTTATT TTTCTAAATA 3420
CATTCAAATA TGTATCCGCT CATGAGACAA TAACCCTGAT AAATGCTTCA ATAATATTGA 3480
AAAAGGAAGA GTATGAGTAT TCAACATTTC CGTGTCGCCC TTATTCCCTT TTTTGCGGCA 3540
TTTTGCCTTC CTGTTTTTGC TCACCCAGAA ACGCTGGTGA AAGTAAAAGA TGCTGAAGAT 3600

CAGTTGGGTG CACGAGTGGG TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG 3660
AGTTTTCGCC CCGAAGAACG TTTTCCAATG ATGAGCACTT TTAAAGTTCT GCTATGTGGC 3720
GCGGTATTAT CCCGTGTTGA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT ACACTATTCT 3780
CAGAATGACT TGGTTGAGTA CTCACCAGTC ACAGAAAAGC ATCTTACGGA TGGCATGACA 3840
GTAAGAGAAT TATGCAGTGC TGCCATAACC ATGAGTGATA ACACTGCGGC CAACTTACTT 3900
CTGACAACGA TCGGAGGACC GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT 3960
GTAACTCGCC TTGATCGTTG GGAACCGGAG CTGAATGAAG CCATACCAAA CGACGAGCGT 4020
GACACCACGA TGCCTGTAGC AATGGCAACA ACGTTGCGCA AACTATTAAC TGGCGAACTA 4080
CTTACTCTAG CTTCCCGGCA ACAATTAATA GACTGGATGG AGGCGGATAA AGTTGCAGGA 4140
CCACTTCTGC GCTCGGCCCT TCCGGCTGGC TGGTTTATTG CTGATAAATC TGGAGCCGGT 4200
GAGCGTGGGT CTCGCGGTAT CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC 4260
GTAGTTATCT ACACGACGGG GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT 4320
GAGATAGGTG CCTCACTGAT TAAGCATTGG TAACTGTCAG ACCAAGTTTA CTCATATATA 4380
CTTTAGATTG ATTTACGCGC CCTGTAGCGG CGCATTAAGC GCGGCGGGTG TGGTGGTTAC 4440
GCGCAGCGTG ACCGCTACAC TTGCCAGCGC CCTAGCGCCC GCTCCTTTCG CTTTCTTCCC 4500
TTCCTTTCTC GCCACGTTCG CCGGCTTTCC CCGTCAAGCT CTAAATCGGG GGCTCCCTTT 4560
AGGGTTCCGA TTTAGTGCTT TACGGCACCT CGACCCCAAA AAACTTGATT TGGGTGATGG 4620
TTCACGTAGT GGGCCATCGC CCTGATAGAC GGTTTTTCGC CCTTTGACGT TGGAGTCCAC 4680
GTTCTTTAAT AGTGGACTCT TGTTCCAAAC TTGAACAACA CTCAACCCTA TCTCGGGCTA 4740
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AGGTGAAGAT CCTTTTTGAT AATCTCATGA CCAAAATCCC TTAACGTGAG TTTTCGTTCC 4920
ACTGAGCGTC AGACCCCGTA GAAAAGATCA AAGGATCTTC TTGAGATCCT TTTTTTCTGC 4980
GCGTAATCTG CTGCTTGCAA ACAAAAAAAC CACCGCTACC AGCGGTGGTT TGTTTGCCGG 5040
ATCAAGAGCT ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG CAGATACCAA 5100
ATACTGTCCT TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT GTAGCACCGC 5160
CTACATACCT CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGGC GATAAGTCGT 5220
GTCTTACCGG GTTGGACTCA AGACGATAGT TACCGGATAA GGCGCAGCGG TCGGGCTGAA 5280
CGGGGGGTTC GTGCACACAG CCCAGCTTGG AGCGAACGAC CTACACCGAA CTGAGATACC 5340
TACAGCGTGA GCTATGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC 5400
CGGTAAGCGG CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT 5460
GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA TTTTTGTGAT 5520
GCTCGTCAGG GGGGCGGAGC CTATGGAAAA ACGCCAGCAA CGCGGCCTTT TTACGGTTCC 5580
TGGCCTTTTG CTGGCCTTTT GCTCACATGT TCTTTCCTGC GTTATCCCCT GATTCTGTGG 5640

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GCAGCGAGTC AGTGAGCGAG GAAGCGGAAG AGCGCCTGAT GCGGTATTTT CTCCTTACGC    5760
ATCTGTGCGG TATTTCACAC CGCATAGGGT CATGGCTGCG CCCCGACACC CGCCAACACC    5820
CGCTGACGCG CCCTGACGGG CTTGTCTGCT CCCGGCATCC GCTTACAGAC AAGCTGTGAC    5880
CGTCTCCGGG AGCTGCATGT GTCAGAGGTT TTCACCGTCA TCACCGAAAC GCGCGAGGCA    5940
GCAAGGAGAT GGCGCCCAAC AGTCCCCCGG CCACGGGGCC TGCCACCATA CCCACGCCGA    6000
AACAAGCGCT CATGAGCCCG AAGTGGCGAG CCCGATCTTC CCCATCGGTG ATGTCGGCGA    6060
TATAGGCGCC AGCAACCGCA CCTGTGGCGC CGGTGATGCC GGCCACGATG CGTCCGGCGT    6120
AGAGGATCTG CTCATGTTTG ACAGCTTATC                                     6150
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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 843 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(vii) IMMEDIATE SOURCE:
(B) CLONE: t-recE

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..843
(D) OTHER INFORMATION:/product= "t-recE"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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ATG GAT CCC GTA ATC GTA GAA GAC ATA GAG CCA GGT ATT TAT TAC GGA      48
Met Asp Pro Val Ile Val Glu Asp Ile Glu Pro Gly Ile Tyr Tyr Gly
  1               5                  10                  15

ATT TCG AAT GAG AAT TAC CAC GCG GGT CCC GGT ATC AGT AAG TCT CAG      96
Ile Ser Asn Glu Asn Tyr His Ala Gly Pro Gly Ile Ser Lys Ser Gln
             20                  25                  30

CTC GAT GAC ATT GCT GAT ACT CCG GCA CTA TAT TTG TGG CGT AAA AAT     144
Leu Asp Asp Ile Ala Asp Thr Pro Ala Leu Tyr Leu Trp Arg Lys Asn
         35                  40                  45

GCC CCC GTG GAC ACC ACA AAG ACA AAA ACG CTC GAT TTA GGA ACT GCT     192
Ala Pro Val Asp Thr Thr Lys Thr Lys Thr Leu Asp Leu Gly Thr Ala
     50                  55                  60

TTC CAC TGC CGG GTA CTT GAA CCG GAA GAA TTC AGT AAC CGC TTT ATC     240
Phe His Cys Arg Val Leu Glu Pro Glu Glu Phe Ser Asn Arg Phe Ile
 65                  70                  75                  80

GTA GCA CCT GAA TTT AAC CGC CGT ACA AAC GCC GGA AAA GAA GAA GAG     288
Val Ala Pro Glu Phe Asn Arg Arg Thr Asn Ala Gly Lys Glu Glu Glu
                 85                  90                  95

AAA GCG TTT CTG ATG GAA TGC GCA AGC ACA GGA AAA ACG GTT ATC ACT     336
Lys Ala Phe Leu Met Glu Cys Ala Ser Thr Gly Lys Thr Val Ile Thr
            100                 105                 110
```

GCG GAA GAA GGC CGG AAA ATT GAA CTC ATG TAT CAA AGC GTT ATG GCT 384
Ala Glu Glu Gly Arg Lys Ile Glu Leu Met Tyr Gln Ser Val Met Ala
115 120 125

TTG CCG CTG GGG CAA TGG CTT GTT GAA AGC GCC GGA CAC GCT GAA TCA 432
Leu Pro Leu Gly Gln Trp Leu Val Glu Ser Ala Gly His Ala Glu Ser
130 135 140

TCA ATT TAC TGG GAA GAT CCT GAA ACA GGA ATT TTG TGT CGG TGC CGT 480
Ser Ile Tyr Trp Glu Asp Pro Glu Thr Gly Ile Leu Cys Arg Cys Arg
145 150 155 160

CCG GAC AAA ATT ATC CCT GAA TTT CAC TGG ATC ATG GAC GTG AAA ACT 528
Pro Asp Lys Ile Ile Pro Glu Phe His Trp Ile Met Asp Val Lys Thr
165 170 175

ACG GCG GAT ATT CAA CGA TTC AAA ACC GCT TAT TAC GAC TAC CGC TAT 576
Thr Ala Asp Ile Gln Arg Phe Lys Thr Ala Tyr Tyr Asp Tyr Arg Tyr
180 185 190

CAC GTT CAG GAT GCA TTC TAC AGT GAC GGT TAT GAA GCA CAG TTT GGA 624
His Val Gln Asp Ala Phe Tyr Ser Asp Gly Tyr Glu Ala Gln Phe Gly
195 200 205

GTG CAG CCA ACT TTC GTT TTT CTG GTT GCC AGC ACA ACT ATT GAA TGC 672
Val Gln Pro Thr Phe Val Phe Leu Val Ala Ser Thr Thr Ile Glu Cys
210 215 220

GGA CGT TAT CCG GTT GAA ATT TTC ATG ATG GGC GAA GAA GCA AAA CTG 720
Gly Arg Tyr Pro Val Glu Ile Phe Met Met Gly Glu Glu Ala Lys Leu
225 230 235 240

GCA GGT CAA CAG GAA TAT CAC CGC AAT CTG CGA ACC CTG TCT GAC TGC 768
Ala Gly Gln Gln Glu Tyr His Arg Asn Leu Arg Thr Leu Ser Asp Cys
245 250 255

CTG AAT ACC GAT GAA TGG CCA GCT ATT AAG ACA TTA TCA CTG CCC CGC 816
Leu Asn Thr Asp Glu Trp Pro Ala Ile Lys Thr Leu Ser Leu Pro Arg
260 265 270

TGG GCT AAG GAA TAT GCA AAT GAC TAA 843
Trp Ala Lys Glu Tyr Ala Asn Asp *
275 280

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 281 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Met Asp Pro Val Ile Val Glu Asp Ile Glu Pro Gly Ile Tyr Tyr Gly
1 5 10 15

Ile Ser Asn Glu Asn Tyr His Ala Gly Pro Gly Ile Ser Lys Ser Gln
20 25 30

Leu Asp Asp Ile Ala Asp Thr Pro Ala Leu Tyr Leu Trp Arg Lys Asn
35 40 45

Ala Pro Val Asp Thr Thr Lys Thr Lys Thr Leu Asp Leu Gly Thr Ala
50 55 60

```
Phe His Cys Arg Val Leu Glu Pro Glu Glu Phe Ser Asn Arg Phe Ile
 65                  70                  75                  80

Val Ala Pro Glu Phe Asn Arg Arg Thr Asn Ala Gly Lys Glu Glu Glu
                 85                  90                  95

Lys Ala Phe Leu Met Glu Cys Ala Ser Thr Gly Lys Thr Val Ile Thr
            100                 105                 110

Ala Glu Glu Gly Arg Lys Ile Glu Leu Met Tyr Gln Ser Val Met Ala
        115                 120                 125

Leu Pro Leu Gly Gln Trp Leu Val Glu Ser Ala Gly His Ala Glu Ser
    130                 135                 140

Ser Ile Tyr Trp Glu Asp Pro Glu Thr Gly Ile Leu Cys Arg Cys Arg
145                 150                 155                 160

Pro Asp Lys Ile Ile Pro Glu Phe His Trp Ile Met Asp Val Lys Thr
                165                 170                 175

Thr Ala Asp Ile Gln Arg Phe Lys Thr Ala Tyr Tyr Asp Tyr Arg Tyr
            180                 185                 190

His Val Gln Asp Ala Phe Tyr Ser Asp Gly Tyr Glu Ala Gln Phe Gly
        195                 200                 205

Val Gln Pro Thr Phe Val Phe Leu Val Ala Ser Thr Thr Ile Glu Cys
    210                 215                 220

Gly Arg Tyr Pro Val Glu Ile Phe Met Met Gly Glu Glu Ala Lys Leu
225                 230                 235                 240

Ala Gly Gln Gln Glu Tyr His Arg Asn Leu Arg Thr Leu Ser Asp Cys
                245                 250                 255

Leu Asn Thr Asp Glu Trp Pro Ala Ile Lys Thr Leu Ser Leu Pro Arg
            260                 265                 270

Trp Ala Lys Glu Tyr Ala Asn Asp  *
        275                 280
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 810 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(vii) IMMEDIATE SOURCE:
(B) CLONE: recT

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..810
(D) OTHER INFORMATION:/product= "recT"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
ATG ACT AAG CAA CCA CCA ATC GCA AAA GCC GAT CTG CAA AAA ACT CAG      48
Met Thr Lys Gln Pro Pro Ile Ala Lys Ala Asp Leu Gln Lys Thr Gln
        285                 290                 295
```

GGA AAC CGT GCA CCA GCA GCA GTT AAA AAT AGC GAC GTG ATT AGT TTT 96
Gly Asn Arg Ala Pro Ala Ala Val Lys Asn Ser Asp Val Ile Ser Phe
300 305 310

ATT AAC CAG CCA TCA ATG AAA GAG CAA CTG GCA GCA GCT CTT CCA CGC 144
Ile Asn Gln Pro Ser Met Lys Glu Gln Leu Ala Ala Ala Leu Pro Arg
315 320 325

CAT ATG ACG GCT GAA CGT ATG ATC CGT ATC GCC ACC ACA GAA ATT CGT 192
His Met Thr Ala Glu Arg Met Ile Arg Ile Ala Thr Thr Glu Ile Arg
330 335 340 345

AAA GTT CCG GCG TTA GGA AAC TGT GAC ACT ATG AGT TTT GTC AGT GCG 240
Lys Val Pro Ala Leu Gly Asn Cys Asp Thr Met Ser Phe Val Ser Ala
350 355 360

ATC GTA CAG TGT TCA CAG CTC GGA CTT GAG CCA GGT AGC GCC CTC GGT 288
Ile Val Gln Cys Ser Gln Leu Gly Leu Glu Pro Gly Ser Ala Leu Gly
365 370 375

CAT GCA TAT TTA CTG CCT TTT GGT AAT AAA AAC GAA AAG AGC GGT AAA 336
His Ala Tyr Leu Leu Pro Phe Gly Asn Lys Asn Glu Lys Ser Gly Lys
380 385 390

AAG AAC GTT CAG CTA ATC ATT GGC TAT CGC GGC ATG ATT GAT CTG GCT 384
Lys Asn Val Gln Leu Ile Ile Gly Tyr Arg Gly Met Ile Asp Leu Ala
395 400 405

CGC CGT TCT GGT CAA ATC GCC AGC CTG TCA GCC CGT GTT GTC CGT GAA 432
Arg Arg Ser Gly Gln Ile Ala Ser Leu Ser Ala Arg Val Val Arg Glu
410 415 420 425

GGT GAC GAG TTT AGC TTC GAA TTT GGC CTT GAT GAA AAG TTA ATA CAC 480
Gly Asp Glu Phe Ser Phe Glu Phe Gly Leu Asp Glu Lys Leu Ile His
430 435 440

CGC CCG GGA GAA AAC GAA GAT GCC CCG GTT ACC CAC GTC TAT GCT GTC 528
Arg Pro Gly Glu Asn Glu Asp Ala Pro Val Thr His Val Tyr Ala Val
445 450 455

GCA AGA CTG AAA GAC GGA GGT ACT CAG TTT GAA GTT ATG ACG CGC AAA 576
Ala Arg Leu Lys Asp Gly Gly Thr Gln Phe Glu Val Met Thr Arg Lys
460 465 470

CAG ATT GAG CTG GTG CGC AGC CTG AGT AAA GCT GGT AAT AAC GGG CCG 624
Gln Ile Glu Leu Val Arg Ser Leu Ser Lys Ala Gly Asn Asn Gly Pro
475 480 485

TGG GTA ACT CAC TGG GAA GAA ATG GCA AAG AAA ACG GCT ATT CGT CGC 672
Trp Val Thr His Trp Glu Glu Met Ala Lys Lys Thr Ala Ile Arg Arg
490 495 500 505

CTG TTC AAA TAT TTG CCC GTA TCA ATT GAG ATC CAG CGT GCA GTA TCA 720
Leu Phe Lys Tyr Leu Pro Val Ser Ile Glu Ile Gln Arg Ala Val Ser
510 515 520

ATG GAT GAA AAG GAA CCA CTG ACA ATC GAT CCT GCA GAT TCC TCT GTA 768
Met Asp Glu Lys Glu Pro Leu Thr Ile Asp Pro Ala Asp Ser Ser Val
525 530 535

TTA ACC GGG GAA TAC AGT GTA ATC GAT AAT TCA GAG GAA TAG 810
Leu Thr Gly Glu Tyr Ser Val Ile Asp Asn Ser Glu Glu *
540 545 550

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 270 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
Met Thr Lys Gln Pro Pro Ile Ala Lys Ala Asp Leu Gln Lys Thr Gln
1               5                   10                  15

Gly Asn Arg Ala Pro Ala Ala Val Lys Asn Ser Asp Val Ile Ser Phe
            20                  25                  30

Ile Asn Gln Pro Ser Met Lys Glu Gln Leu Ala Ala Ala Leu Pro Arg
        35                  40                  45

His Met Thr Ala Glu Arg Met Ile Arg Ile Ala Thr Thr Glu Ile Arg
    50                  55                  60

Lys Val Pro Ala Leu Gly Asn Cys Asp Thr Met Ser Phe Val Ser Ala
65                  70                  75                  80

Ile Val Gln Cys Ser Gln Leu Gly Leu Glu Pro Gly Ser Ala Leu Gly
                85                  90                  95

His Ala Tyr Leu Leu Pro Phe Gly Asn Lys Asn Glu Lys Ser Gly Lys
            100                 105                 110

Lys Asn Val Gln Leu Ile Ile Gly Tyr Arg Gly Met Ile Asp Leu Ala
        115                 120                 125

Arg Arg Ser Gly Gln Ile Ala Ser Leu Ser Ala Arg Val Val Arg Glu
    130                 135                 140

Gly Asp Glu Phe Ser Phe Glu Phe Gly Leu Asp Glu Lys Leu Ile His
145                 150                 155                 160

Arg Pro Gly Glu Asn Glu Asp Ala Pro Val Thr His Val Tyr Ala Val
                165                 170                 175

Ala Arg Leu Lys Asp Gly Gly Thr Gln Phe Glu Val Met Thr Arg Lys
            180                 185                 190

Gln Ile Glu Leu Val Arg Ser Leu Ser Lys Ala Gly Asn Asn Gly Pro
        195                 200                 205

Trp Val Thr His Trp Glu Glu Met Ala Lys Lys Thr Ala Ile Arg Arg
    210                 215                 220

Leu Phe Lys Tyr Leu Pro Val Ser Ile Glu Ile Gln Arg Ala Val Ser
225                 230                 235                 240

Met Asp Glu Lys Glu Pro Leu Thr Ile Asp Pro Ala Asp Ser Ser Val
                245                 250                 255

Leu Thr Gly Glu Tyr Ser Val Ile Asp Asn Ser Glu Glu  *
            260                 265                      270
```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 876 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(vii) IMMEDIATE SOURCE:
(B) CLONE: araC

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:complement (1..876)
(D) OTHER INFORMATION:/product= "araC"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
TGACAACTTG ACGGCTACAT CATTCACTTT TTCTTCACAA CCGGCACGGA ACTCGCTCGG   60
GCTGGCCCCG GTGCATTTTT TAAATACCCG CGAGAAATAG AGTTGATCGT CAAAACCAAC  120
ATTGCGACCG ACGGTGGCGA TAGGCATCCG GGTGGTGCTC AAAAGCAGCT TCGCCTGGCT  180
GATACGTTGG TCCTCGCGCC AGCTTAAGAC GCTAATCCCT AACTGCTGGC GGAAAAGATG  240
TGACAGACGC GACGGCGACA AGCAAACATG CTGTGCGACG CTGGCGATAT CAAAATTGCT  300
GTCTGCCAGG TGATCGCTGA TGTACTGACA AGCCTCGCGT ACCCGATTAT CCATCGGTGG  360
ATGGAGCGAC TCGTTAATCG CTTCCATGCG CCGCAGTAAC AATTGCTCAA GCAGATTTAT  420
CGCCAGCAGC TCCGAATAGC GCCCTTCCCC TTGCCCGGCG TTAATGATTT GCCCAAACAG  480
GTCGCTGAAA TGCGGCTGGT GCGCTTCATC CGGGCGAAAG AACCCCGTAT TGGCAAATAT  540
TGACGGCCAG TTAAGCCATT CATGCCAGTA GGCGCGCGGA CGAAAGTAAA CCCACTGGTG  600
ATACCATTCG CGAGCCTCCG GATGACGACC GTAGTGATGA ATCTCTCCTG GCGGGAACAG  660
CAAAATATCA CCCGGTCGGC AAACAAATTC TCGTCCCTGA TTTTTCACCA CCCCCTGACC  720
GCGAATGGTG AGATTGAGAA TATAACCTTT CATTCCCAGC GGTCGGTCGA TAAAAAAATC  780
GAGATAACCG TTGGCCTCAA TCGGCGTTAA ACCCGCCACC AGATGGGCAT TAAACGAGTA  840
TCCCGGCAGC AGGGGATCAT TTTGCGCTTC AGCCAT                             876
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 292 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```
Met Ala Glu Ala Gln Asn Asp Pro Leu Leu Pro Gly Tyr Ser Phe Asn
  1               5                  10                  15

Ala His Leu Val Ala Gly Leu Thr Pro Ile Glu Ala Asn Gly Tyr Leu
            20                  25                  30
```

```
Asp Phe Phe Ile Asp Arg Pro Leu Gly Met Lys Gly Tyr Ile Leu Asn
         35                  40                      45

Leu Thr Ile Arg Gly Gln Gly Val Val Lys Asn Gln Gly Arg Glu Phe
     50                      55                  60

Val Cys Arg Pro Gly Asp Ile Leu Leu Phe Pro Pro Gly Glu Ile His
 65                  70                  75                  80

His Tyr Gly Arg His Pro Glu Ala Arg Glu Trp Tyr His Gln Trp Val
                 85                  90                  95

Tyr Phe Arg Pro Arg Ala Tyr Trp His Glu Trp Leu Asn Trp Pro Ser
            100                 105                 110

Ile Phe Ala Asn Thr Gly Phe Phe Arg Pro Asp Glu Ala His Gln Pro
        115                 120                 125

His Phe Ser Asp Leu Phe Gly Gln Ile Ile Asn Ala Gly Gln Gly Glu
    130                 135                 140

Gly Arg Tyr Ser Glu Leu Leu Ala Ile Asn Leu Leu Glu Gln Leu Leu
145                 150                 155                 160

Leu Arg Arg Met Glu Ala Ile Asn Glu Ser Leu His Pro Pro Met Asp
                165                 170                 175

Asn Arg Val Arg Glu Ala Cys Gln Tyr Ile Ser Asp His Leu Ala Asp
            180                 185                 190

Ser Asn Phe Asp Ile Ala Ser Val Ala Gln His Val Cys Leu Ser Pro
        195                 200                 205

Ser Arg Leu Ser His Leu Phe Arg Gln Gln Leu Gly Ile Ser Val Leu
    210                 215                 220

Ser Trp Arg Glu Asp Gln Arg Ile Ser Gln Ala Lys Leu Leu Leu Ser
225                 230                 235                 240

Thr Thr Arg Met Pro Ile Ala Thr Val Gly Arg Asn Val Gly Phe Asp
                245                 250                 255

Asp Gln Leu Tyr Phe Ser Arg Val Phe Lys Lys Cys Thr Gly Ala Ser
            260                 265                 270

Pro Ser Glu Phe Arg Ala Gly Cys Glu Glu Lys Val Asn Asp Val Ala
        275                 280                 285

Val Lys Leu Ser
    290
```

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 861 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(vii) IMMEDIATE SOURCE:
(B) CLONE: bla

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..861
(D) OTHER INFORMATION:/product= "bla"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
ATG AGT ATT CAA CAT TTC CGT GTC GCC CTT ATT CCC TTT TTT GCG GCA      48
Met Ser Ile Gln His Phe Arg Val Ala Leu Ile Pro Phe Phe Ala Ala
        295                 300                 305

TTT TGC CTT CCT GTT TTT GCT CAC CCA GAA ACG CTG GTG AAA GTA AAA      96
Phe Cys Leu Pro Val Phe Ala His Pro Glu Thr Leu Val Lys Val Lys
    310                 315                 320

GAT GCT GAA GAT CAG TTG GGT GCA CGA GTG GGT TAC ATC GAA CTG GAT     144
Asp Ala Glu Asp Gln Leu Gly Ala Arg Val Gly Tyr Ile Glu Leu Asp
325                 330                 335                 340

CTC AAC AGC GGT AAG ATC CTT GAG AGT TTT CGC CCC GAA GAA CGT TTT     192
Leu Asn Ser Gly Lys Ile Leu Glu Ser Phe Arg Pro Glu Glu Arg Phe
                345                 350                 355

CCA ATG ATG AGC ACT TTT AAA GTT CTG CTA TGT GGC GCG GTA TTA TCC     240
Pro Met Met Ser Thr Phe Lys Val Leu Leu Cys Gly Ala Val Leu Ser
            360                 365                 370

CGT GTT GAC GCC GGG CAA GAG CAA CTC GGT CGC CGC ATA CAC TAT TCT     288
Arg Val Asp Ala Gly Gln Glu Gln Leu Gly Arg Arg Ile His Tyr Ser
        375                 380                 385

CAG AAT GAC TTG GTT GAG TAC TCA CCA GTC ACA GAA AAG CAT CTT ACG     336
Gln Asn Asp Leu Val Glu Tyr Ser Pro Val Thr Glu Lys His Leu Thr
    390                 395                 400

GAT GGC ATG ACA GTA AGA GAA TTA TGC AGT GCT GCC ATA ACC ATG AGT     384
Asp Gly Met Thr Val Arg Glu Leu Cys Ser Ala Ala Ile Thr Met Ser
405                 410                 415                 420

GAT AAC ACT GCG GCC AAC TTA CTT CTG ACA ACG ATC GGA GGA CCG AAG     432
Asp Asn Thr Ala Ala Asn Leu Leu Leu Thr Thr Ile Gly Gly Pro Lys
                425                 430                 435

GAG CTA ACC GCT TTT TTG CAC AAC ATG GGG GAT CAT GTA ACT CGC CTT     480
Glu Leu Thr Ala Phe Leu His Asn Met Gly Asp His Val Thr Arg Leu
            440                 445                 450

GAT CGT TGG GAA CCG GAG CTG AAT GAA GCC ATA CCA AAC GAC GAG CGT     528
Asp Arg Trp Glu Pro Glu Leu Asn Glu Ala Ile Pro Asn Asp Glu Arg
        455                 460                 465

GAC ACC ACG ATG CCT GTA GCA ATG GCA ACA ACG TTG CGC AAA CTA TTA     576
Asp Thr Thr Met Pro Val Ala Met Ala Thr Thr Leu Arg Lys Leu Leu
    470                 475                 480

ACT GGC GAA CTA CTT ACT CTA GCT TCC CGG CAA CAA TTA ATA GAC TGG     624
Thr Gly Glu Leu Leu Thr Leu Ala Ser Arg Gln Gln Leu Ile Asp Trp
485                 490                 495                 500

ATG GAG GCG GAT AAA GTT GCA GGA CCA CTT CTG CGC TCG GCC CTT CCG     672
Met Glu Ala Asp Lys Val Ala Gly Pro Leu Leu Arg Ser Ala Leu Pro
                505                 510                 515

GCT GGC TGG TTT ATT GCT GAT AAA TCT GGA GCC GGT GAG CGT GGG TCT     720
Ala Gly Trp Phe Ile Ala Asp Lys Ser Gly Ala Gly Glu Arg Gly Ser
            520                 525                 530
```

```
CGC GGT ATC ATT GCA GCA CTG GGG CCA GAT GGT AAG CCC TCC CGT ATC      768
Arg Gly Ile Ile Ala Ala Leu Gly Pro Asp Gly Lys Pro Ser Arg Ile
        535                 540                 545

GTA GTT ATC TAC ACG ACG GGG AGT CAG GCA ACT ATG GAT GAA CGA AAT      816
Val Val Ile Tyr Thr Thr Gly Ser Gln Ala Thr Met Asp Glu Arg Asn
    550                 555                 560

AGA CAG ATC GCT GAG ATA GGT GCC TCA CTG ATT AAG CAT TGG TAA          861
Arg Gln Ile Ala Glu Ile Gly Ala Ser Leu Ile Lys His Trp  *
565                 570                 575
```

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 287 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
Met Ser Ile Gln His Phe Arg Val Ala Leu Ile Pro Phe Phe Ala Ala
1               5                   10                  15

Phe Cys Leu Pro Val Phe Ala His Pro Glu Thr Leu Val Lys Val Lys
            20                  25                  30

Asp Ala Glu Asp Gln Leu Gly Ala Arg Val Gly Tyr Ile Glu Leu Asp
        35                  40                  45

Leu Asn Ser Gly Lys Ile Leu Glu Ser Phe Arg Pro Glu Glu Arg Phe
    50                  55                  60

Pro Met Met Ser Thr Phe Lys Val Leu Leu Cys Gly Ala Val Leu Ser
65                  70                  75                  80

Arg Val Asp Ala Gly Gln Glu Gln Leu Gly Arg Arg Ile His Tyr Ser
                85                  90                  95

Gln Asn Asp Leu Val Glu Tyr Ser Pro Val Thr Glu Lys His Leu Thr
            100                 105                 110

Asp Gly Met Thr Val Arg Glu Leu Cys Ser Ala Ala Ile Thr Met Ser
        115                 120                 125

Asp Asn Thr Ala Ala Asn Leu Leu Leu Thr Thr Ile Gly Gly Pro Lys
    130                 135                 140

Glu Leu Thr Ala Phe Leu His Asn Met Gly Asp His Val Thr Arg Leu
145                 150                 155                 160

Asp Arg Trp Glu Pro Glu Leu Asn Glu Ala Ile Pro Asn Asp Glu Arg
                165                 170                 175

Asp Thr Thr Met Pro Val Ala Met Ala Thr Thr Leu Arg Lys Leu Leu
            180                 185                 190

Thr Gly Glu Leu Leu Thr Leu Ala Ser Arg Gln Gln Leu Ile Asp Trp
        195                 200                 205

Met Glu Ala Asp Lys Val Ala Gly Pro Leu Leu Arg Ser Ala Leu Pro
    210                 215                 220

Ala Gly Trp Phe Ile Ala Asp Lys Ser Gly Ala Gly Glu Arg Gly Ser
```

225 230 235 240

Arg Gly Ile Ile Ala Ala Leu Gly Pro Asp Gly Lys Pro Ser Arg Ile
245 250 255

Val Val Ile Tyr Thr Thr Gly Ser Gln Ala Thr Met Asp Glu Arg Asn
260 265 270

Arg Gln Ile Ala Glu Ile Gly Ala Ser Leu Ile Lys His Trp *
275 280 285

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7195 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(vii) IMMEDIATE SOURCE:
(B) CLONE: pBAD-ETgamma

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION:3588..4004
(D) OTHER INFORMATION:/product= "red gamma"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

ATCGATGCAT AATGTGCCTG TCAAATGGAC GAAGCAGGGA TTCTGCAAAC CCTATGCTAC 60

TCCGTCAAGC CGTCAATTGT CTGATTCGTT ACCAATTATG ACAACTTGAC GGCTACATCA 120

TTCACTTTTT CTTCACAACC GGCACGGAAC TCGCTCGGGC TGGCCCCGGT GCATTTTTTA 180

AATACCCGCG AGAAATAGAG TTGATCGTCA AAACCAACAT TGCGACCGAC GGTGGCGATA 240

GGCATCCGGG TGGTGCTCAA AAGCAGCTTC GCCTGGCTGA TACGTTGGTC CTCGCGCCAG 300

CTTAAGACGC TAATCCCTAA CTGCTGGCGG AAAAGATGTG ACAGACGCGA CGGCGACAAG 360

CAAACATGCT GTGCGACGCT GGCGATATCA AAATTGCTGT CTGCCAGGTG ATCGCTGATG 420

TACTGACAAG CCTCGCGTAC CCGATTATCC ATCGGTGGAT GGAGCGACTC GTTAATCGCT 480

TCCATGCGCC GCAGTAACAA TTGCTCAAGC AGATTTATCG CCAGCAGCTC CGAATAGCGC 540

CCTTCCCCTT GCCCGGCGTT AATGATTTGC CCAAACAGGT CGCTGAAATG CGGCTGGTGC 600

GCTTCATCCG GGCGAAAGAA CCCCGTATTG GCAAATATTG ACGGCCAGTT AAGCCATTCA 660

TGCCAGTAGG CGCGCGGACG AAAGTAAACC CACTGGTGAT ACCATTCGCG AGCCTCCGGA 720

TGACGACCGT AGTGATGAAT CTCTCCTGGC GGGAACAGCA AAATATCACC CGGTCGGCAA 780

ACAAATTCTC GTCCCTGATT TTTCACCACC CCCTGACCGC GAATGGTGAG ATTGAGAATA 840

TAACCTTTCA TTCCCAGCGG TCGGTCGATA AAAAAATCGA GATAACCGTT GGCCTCAATC 900

GGCGTTAAAC CCGCCACCAG ATGGGCATTA AACGAGTATC CCGGCAGCAG GGGATCATTT 960

TGCGCTTCAG CCATACTTTT CATACTCCCG CCATTCAGAG AAGAAACCAA TTGTCCATAT 1020

TGCATCAGAC ATTGCCGTCA CTGCGTCTTT TACTGGCTCT TCTCGCTAAC CAAACCGGTA 1080
ACCCCGCTTA TTAAAAGCAT TCTGTAACAA AGCGGGACCA AAGCCATGAC AAAAACGCGT 1140
AACAAAAGTG TCTATAATCA CGGCAGAAAA GTCCACATTG ATTATTTGCA CGGCGTCACA 1200
CTTTGCTATG CCATAGCATT TTTATCCATA AGATTAGCGG ATCCTACCTG ACGCTTTTTA 1260
TCGCAACTCT CTACTGTTTC TCCATACCCG TTTTTTTGGG CTAGCAGGAG GAATTCACCA 1320
TGGATCCCGT AATCGTAGAA GACATAGAGC CAGGTATTTA TTACGGAATT TCGAATGAGA 1380
ATTACCACGC GGGTCCCGGT ATCAGTAAGT CTCAGCTCGA TGACATTGCT GATACTCCGG 1440
CACTATATTT GTGGCGTAAA AATGCCCCCG TGGACACCAC AAAGACAAAA ACGCTCGATT 1500
TAGGAACTGC TTTCCACTGC CGGGTACTTG AACCGGAAGA ATTCAGTAAC CGCTTTATCG 1560
TAGCACCTGA ATTTAACCGC CGTACAAACG CCGGAAAAGA AGAAGAGAAA GCGTTTCTGA 1620
TGGAATGCGC AAGCACAGGA AAAACGGTTA TCACTGCGGA AGAAGGCCGG AAAATTGAAC 1680
TCATGTATCA AAGCGTTATG GCTTTGCCGC TGGGGCAATG GCTTGTTGAA AGCGCCGGAC 1740
ACGCTGAATC ATCAATTTAC TGGGAAGATC CTGAAACAGG AATTTTGTGT CGGTGCCGTC 1800
CGGACAAAAT TATCCCTGAA TTTCACTGGA TCATGGACGT GAAAACTACG GCGGATATTC 1860
AACGATTCAA AACCGCTTAT TACGACTACC GCTATCACGT TCAGGATGCA TTCTACAGTG 1920
ACGGTTATGA AGCACAGTTT GGAGTGCAGC CAACTTTCGT TTTTCTGGTT GCCAGCACAA 1980
CTATTGAATG CGGACGTTAT CCGGTTGAAA TTTTCATGAT GGGCGAAGAA GCAAAACTGG 2040
CAGGTCAACA GGAATATCAC CGCAATCTGC GAACCCTGTC TGACTGCCTG AATACCGATG 2100
AATGGCCAGC TATTAAGACA TTATCACTGC CCCGCTGGGC TAAGGAATAT GCAAATGACT 2160
AGATCTCGAG GTACCCGAGC ACGTGTTGAC AATTAATCAT CGGCATAGTA TATCGGCATA 2220
GTATAATACG ACAAGGTGAG GAACTAAACC ATGGCTAAGC AACCACCAAT CGCAAAAGCC 2280
GATCTGCAAA AACTCAGGG AAACCGTGCA CCAGCAGCAG TTAAAAATAG CGACGTGATT 2340
AGTTTTATTA ACCAGCCATC AATGAAAGAG CAACTGGCAG CAGCTCTTCC ACGCCATATG 2400
ACGGCTGAAC GTATGATCCG TATCGCCACC ACAGAAATTC GTAAAGTTCC GGCGTTAGGA 2460
AACTGTGACA CTATGAGTTT TGTCAGTGCG ATCGTACAGT GTTCACAGCT CGGACTTGAG 2520
CCAGGTAGCG CCCTCGGTCA TGCATATTTA CTGCCTTTTG GTAATAAAAA CGAAAAGAGC 2580
GGTAAAAAGA ACGTTCAGCT AATCATTGGC TATCGCGGCA TGATTGATCT GGCTCGCCGT 2640
TCTGGTCAAA TCGCCAGCCT GTCAGCCCGT GTTGTCCGTG AAGGTGACGA GTTTAGCTTC 2700
GAATTTGGCC TTGATGAAAA GTTAATACAC CGCCCGGGAG AAAACGAAGA TGCCCCGGTT 2760
ACCCACGTCT ATGCTGTCGC AAGACTGAAA GACGGAGGTA CTCAGTTTGA AGTTATGACG 2820
CGCAAACAGA TTGAGCTGGT GCGCAGCCTG AGTAAAGCTG GTAATAACGG GCCGTGGGTA 2880
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CCTGCAGATT CCTCTGTATT AACCGGGGAA TACAGTGTAA TCGATAATTC AGAGGAATAG 3060

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TTGGCATTTT CTTTTATTTC TTATCAACAT AAAGGTGAAT CCCATACCTC GAGCTTCACG 3240
CTGCCGCAAG CACTCAGGGC GCAAGGGCTG CTAAAAGGAA GCGGAACACG TAGAAAGCCA 3300
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AAAACGCAAG CGCAAAGAGA AAGCAGGTAG CTTGCAGTGG GCTTACATGG CGATAGCTAG 3420
ACTGGGCGGT TTTATGGACA GCAAGCGAAC CGGAATTGCC AGCTGGGGCG CCCTCTGGTA 3480
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GCAGGGGATC AAGATCTGAT CAAGAGACAG GATGAGGATC GTTTCGCATG GATATTAATA 3600
CTGAAACTGA GATCAAGCAA AAGCATTCAC TAACCCCCTT TCCTGTTTTC CTAATCAGCC 3660
CGGCATTTCG CGGGCGATAT TTTCACAGCT ATTTCAGGAG TTCAGCCATG AACGCTTATT 3720
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AAGAGAAAGA GGCAGAACTG GCAGACGACA TGGAAAAAGG CCTGCCCCAG CACCTGTTTG 3840
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CGTCATCACC GAAACGCGCG AGGCAGCAAG GAGATGGCGC CCAACAGTCC CCCGGCCACG 7020
GGGCCTGCCA CCATACCCAC GCCGAAACAA GCGCTCATGA GCCCGAAGTG GCGAGCCCGA 7080
TCTTCCCCAT CGGTGATGTC GGCGATATAG GCGCCAGCAA CCGCACCTGT GGCGCCGGTG 7140

ATGCCGGCCA CGATGCGTCC GGCGTAGAGG ATCTGCTCAT GTTTGACAGC TTATC 7195

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7010 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(vii) IMMEDIATE SOURCE:
(B) CLONE: pBAD-alpha-beta-gamma

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1320..2000
(D) OTHER INFORMATION:/product= "red alpha"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:2086..2871
(D) OTHER INFORMATION:/product= "red beta"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:3403..3819
(D) OTHER INFORMATION:/product= "red gamma"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

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TCCGTCAAGC CGTCAATTGT CTGATTCGTT ACCAATTATG ACAACTTGAC GGCTACATCA 120
TTCACTTTTT CTTCACAACC GGCACGGAAC TCGCTCGGGC TGGCCCCGGT GCATTTTTTA 180
AATACCCGCG AGAAATAGAG TTGATCGTCA AAACCAACAT TGCGACCGAC GGTGGCGATA 240
GGCATCCGGG TGGTGCTCAA AAGCAGCTTC GCCTGGCTGA TACGTTGGTC CTCGCGCCAG 300
CTTAAGACGC TAATCCCTAA CTGCTGGCGG AAAAGATGTG ACAGACGCGA CGGCGACAAG 360
CAAACATGCT GTGCGACGCT GGCGATATCA AAATTGCTGT CTGCCAGGTG ATCGCTGATG 420
TACTGACAAG CCTCGCGTAC CCGATTATCC ATCGGTGGAT GGAGCGACTC GTTAATCGCT 480
TCCATGCGCC GCAGTAACAA TTGCTCAAGC AGATTTATCG CCAGCAGCTC CGAATAGCGC 540
CCTTCCCCTT GCCCGGCGTT AATGATTTGC CCAAACAGGT CGCTGAAATG CGGCTGGTGC 600
GCTTCATCCG GGCGAAAGAA CCCCGTATTG GCAAATATTG ACGGCCAGTT AAGCCATTCA 660
TGCCAGTAGG CGCGCGGACG AAAGTAAACC CACTGGTGAT ACCATTCGCG AGCCTCCGGA 720
TGACGACCGT AGTGATGAAT CTCTCCTGGC GGGAACAGCA AAATATCACC CGGTCGGCAA 780
ACAAATTCTC GTCCCTGATT TTTCACCACC CCCTGACCGC GAATGGTGAG ATTGAGAATA 840
TAACCTTTCA TTCCCAGCGG TCGGTCGATA AAAAAATCGA GATAACCGTT GGCCTCAATC 900
GGCGTTAAAC CCGCCACCAG ATGGGCATTA AACGAGTATC CCGGCAGCAG GGGATCATTT 960

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TGCGCTTCAG CCATACTTTT CATACTCCCG CCATTCAGAG AAGAAACCAA TTGTCCATAT   1020

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ACCCCGCTTA TTAAAAGCAT TCTGTAACAA AGCGGGACCA AAGCCATGAC AAAAACGCGT   1140

AACAAAAGTG TCTATAATCA CGGCAGAAAA GTCCACATTG ATTATTTGCA CGGCGTCACA   1200

CTTTGCTATG CCATAGCATT TTTATCCATA AGATTAGCGG ATCCTACCTG ACGCTTTTTA   1260

TCGCAACTCT CTACTGTTTC TCCATACCCG TTTTTTTGGG CTAGCAGGAG GAATTCACC    1319

ATG ACA CCG GAC ATT ATC CTG CAG CGT ACC GGG ATC GAT GTG AGA GCT     1367
Met Thr Pro Asp Ile Ile Leu Gln Arg Thr Gly Ile Asp Val Arg Ala
        290                 295                 300

GTC GAA CAG GGG GAT GAT GCG TGG CAC AAA TTA CGG CTC GGC GTC ATC     1415
Val Glu Gln Gly Asp Asp Ala Trp His Lys Leu Arg Leu Gly Val Ile
    305                 310                 315

ACC GCT TCA GAA GTT CAC AAC GTG ATA GCA AAA CCC CGC TCC GGA AAG     1463
Thr Ala Ser Glu Val His Asn Val Ile Ala Lys Pro Arg Ser Gly Lys
320                 325                 330                 335

AAG TGG CCT GAC ATG AAA ATG TCC TAC TTC CAC ACC CTG CTT GCT GAG     1511
Lys Trp Pro Asp Met Lys Met Ser Tyr Phe His Thr Leu Leu Ala Glu
                340                 345                 350

GTT TGC ACC GGT GTG GCT CCG GAA GTT AAC GCT AAA GCA CTG GCC TGG     1559
Val Cys Thr Gly Val Ala Pro Glu Val Asn Ala Lys Ala Leu Ala Trp
            355                 360                 365

GGA AAA CAG TAC GAG AAC GAC GCC AGA ACC CTG TTT GAA TTC ACT TCC     1607
Gly Lys Gln Tyr Glu Asn Asp Ala Arg Thr Leu Phe Glu Phe Thr Ser
        370                 375                 380

GGC GTG AAT GTT ACT GAA TCC CCG ATC ATC TAT CGC GAC GAA AGT ATG     1655
Gly Val Asn Val Thr Glu Ser Pro Ile Ile Tyr Arg Asp Glu Ser Met
    385                 390                 395

CGT ACC GCC TGC TCT CCC GAT GGT TTA TGC AGT GAC GGC AAC GGC CTT     1703
Arg Thr Ala Cys Ser Pro Asp Gly Leu Cys Ser Asp Gly Asn Gly Leu
400                 405                 410                 415

GAA CTG AAA TGC CCG TTT ACC TCC CGG GAT TTC ATG AAG TTC CGG CTC     1751
Glu Leu Lys Cys Pro Phe Thr Ser Arg Asp Phe Met Lys Phe Arg Leu
                420                 425                 430

GGT GGT TTC GAG GCC ATA AAG TCA GCT TAC ATG GCC CAG GTG CAG TAC     1799
Gly Gly Phe Glu Ala Ile Lys Ser Ala Tyr Met Ala Gln Val Gln Tyr
            435                 440                 445

AGC ATG TGG GTG ACG CGA AAA AAT GCC TGG TAC TTT GCC AAC TAT GAC     1847
Ser Met Trp Val Thr Arg Lys Asn Ala Trp Tyr Phe Ala Asn Tyr Asp
        450                 455                 460

CCG CGT ATG AAG CGT GAA GGC CTG CAT TAT GTC GTG ATT GAG CGG GAT     1895
Pro Arg Met Lys Arg Glu Gly Leu His Tyr Val Val Ile Glu Arg Asp
    465                 470                 475

GAA AAG TAC ATG GCG AGT TTT GAC GAG ATC GTG CCG GAG TTC ATC GAA     1943
Glu Lys Tyr Met Ala Ser Phe Asp Glu Ile Val Pro Glu Phe Ile Glu
480                 485                 490                 495

AAA ATG GAC GAG GCA CTG GCT GAA ATT GGT TTT GTA TTT GGG GAG CAA     1991
Lys Met Asp Glu Ala Leu Ala Glu Ile Gly Phe Val Phe Gly Glu Gln
                500                 505                 510
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TGG CGA TAG ATCCGGTACC CGAGCACGTG TTGACAATTA ATCATCGGCA 2040
Trp Arg *

TAGTATATCG GCATAGTATA ATACGACAAG GTGAGGAACT AAACC ATG AGT ACT 2094
Met Ser Thr
1

GCA CTC GCA ACG CTG GCT GGG AAG CTG GCT GAA CGT GTC GGC ATG GAT 2142
Ala Leu Ala Thr Leu Ala Gly Lys Leu Ala Glu Arg Val Gly Met Asp
5 10 15

TCT GTC GAC CCA CAG GAA CTG ATC ACC ACT CTT CGC CAG ACG GCA TTT 2190
Ser Val Asp Pro Gln Glu Leu Ile Thr Thr Leu Arg Gln Thr Ala Phe
20 25 30 35

AAA GGT GAT GCC AGC GAT GCG CAG TTC ATC GCA TTA CTG ATC GTT GCC 2238
Lys Gly Asp Ala Ser Asp Ala Gln Phe Ile Ala Leu Leu Ile Val Ala
40 45 50

AAC CAG TAC GGC CTT AAT CCG TGG ACG AAA GAA ATT TAC GCC TTT CCT 2286
Asn Gln Tyr Gly Leu Asn Pro Trp Thr Lys Glu Ile Tyr Ala Phe Pro
55 60 65

GAT AAG CAG AAT GGC ATC GTT CCG GTG GTG GGC GTT GAT GGC TGG TCC 2334
Asp Lys Gln Asn Gly Ile Val Pro Val Val Gly Val Asp Gly Trp Ser
70 75 80

CGC ATC ATC AAT GAA AAC CAG CAG TTT GAT GGC ATG GAC TTT GAG CAG 2382
Arg Ile Ile Asn Glu Asn Gln Gln Phe Asp Gly Met Asp Phe Glu Gln
85 90 95

GAC AAT GAA TCC TGT ACA TGC CGG ATT TAC CGC AAG GAC CGT AAT CAT 2430
Asp Asn Glu Ser Cys Thr Cys Arg Ile Tyr Arg Lys Asp Arg Asn His
100 105 110 115

CCG ATC TGC GTT ACC GAA TGG ATG GAT GAA TGC CGC CGC GAA CCA TTC 2478
Pro Ile Cys Val Thr Glu Trp Met Asp Glu Cys Arg Arg Glu Pro Phe
120 125 130

AAA ACT CGC GAA GGC AGA GAA ATC ACG GGG CCG TGG CAG TCG CAT CCC 2526
Lys Thr Arg Glu Gly Arg Glu Ile Thr Gly Pro Trp Gln Ser His Pro
135 140 145

AAA CGG ATG TTA CGT CAT AAA GCC ATG ATT CAG TGT GCC CGT CTG GCC 2574
Lys Arg Met Leu Arg His Lys Ala Met Ile Gln Cys Ala Arg Leu Ala
150 155 160

TTC GGA TTT GCT GGT ATC TAT GAC AAG GAT GAA GCC GAG CGC ATT GTC 2622
Phe Gly Phe Ala Gly Ile Tyr Asp Lys Asp Glu Ala Glu Arg Ile Val
165 170 175

GAA AAT ACT GCA TAC ACT GCA GAA CGT CAG CCG GAA CGC GAC ATC ACT 2670
Glu Asn Thr Ala Tyr Thr Ala Glu Arg Gln Pro Glu Arg Asp Ile Thr
180 185 190 195

CCG GTT AAC GAT GAA ACC ATG CAG GAG ATT AAC ACT CTG CTG ATC GCC 2718
Pro Val Asn Asp Glu Thr Met Gln Glu Ile Asn Thr Leu Leu Ile Ala
200 205 210

CTG GAT AAA ACA TGG GAT GAC GAC TTA TTG CCG CTC TGT TCC CAG ATA 2766
Leu Asp Lys Thr Trp Asp Asp Asp Leu Leu Pro Leu Cys Ser Gln Ile
215 220 225

TTT CGC CGC GAC ATT CGT GCA TCG TCA GAA CTG ACA CAG GCC GAA GCA 2814
Phe Arg Arg Asp Ile Arg Ala Ser Ser Glu Leu Thr Gln Ala Glu Ala
230 235 240

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GTA AAA GCT CTT GGA TTC CTG AAA CAG AAA GCC GCA GAG CAG AAG GTG     2862
Val Lys Ala Leu Gly Phe Leu Lys Gln Lys Ala Ala Glu Gln Lys Val
    245                 250                 255

GCA GCA TAG ATCTCGAGAA GCTTCCTGCT GAACATCAAA GGCAAGAAAA             2911
Ala Ala  *
260

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GAATCCCATA CCTCGAGCTT CACGCTGCCG CAAGCACTCA GGGCGCAAGG GCTGCTAAAA   3091

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             Met Asp Ile Asn Thr Glu Thr Glu Ile Lys Gln Lys His
               1               5                  10

TCA CTA ACC CCC TTT CCT GTT TTC CTA ATC AGC CCG GCA TTT CGC GGG     3489
Ser Leu Thr Pro Phe Pro Val Phe Leu Ile Ser Pro Ala Phe Arg Gly
     15                  20                  25

CGA TAT TTT CAC AGC TAT TTC AGG AGT TCA GCC ATG AAC GCT TAT TAC     3537
Arg Tyr Phe His Ser Tyr Phe Arg Ser Ser Ala Met Asn Ala Tyr Tyr
 30                  35                  40                  45

ATT CAG GAT CGT CTT GAG GCT CAG AGC TGG GCG CGT CAC TAC CAG CAG     3585
Ile Gln Asp Arg Leu Glu Ala Gln Ser Trp Ala Arg His Tyr Gln Gln
                 50                  55                  60

CTC GCC CGT GAA GAG AAA GAG GCA GAA CTG GCA GAC GAC ATG GAA AAA     3633
Leu Ala Arg Glu Glu Lys Glu Ala Glu Leu Ala Asp Asp Met Glu Lys
             65                  70                  75

GGC CTG CCC CAG CAC CTG TTT GAA TCG CTA TGC ATC GAT CAT TTG CAA     3681
Gly Leu Pro Gln His Leu Phe Glu Ser Leu Cys Ile Asp His Leu Gln
         80                  85                  90

CGC CAC GGG GCC AGC AAA AAA TCC ATT ACC CGT GCG TTT GAT GAC GAT     3729
Arg His Gly Ala Ser Lys Lys Ser Ile Thr Arg Ala Phe Asp Asp Asp
     95                 100                 105

GTT GAG TTT CAG GAG CGC ATG GCA GAA CAC ATC CGG TAC ATG GTT GAA     3777
Val Glu Phe Gln Glu Arg Met Ala Glu His Ile Arg Tyr Met Val Glu
110                 115                 120                 125

ACC ATT GCT CAC CAC CAG GTT GAT ATT GAT TCA GAG GTA TAA             3819
Thr Ile Ala His His Gln Val Asp Ile Asp Ser Glu Val  *
                130                 135

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GTCCCACCTG ACCCCATGCC GAACTCAGAA GTGAAACGCC GTAGCGCCGA TGGTAGTGTG   3999

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GCTTGTCTGC TCCCGGCATC CGCTTACAGA CAAGCTGTGA CCGTCTCCGG GAGCTGCATG 6759
TGTCAGAGGT TTTCACCGTC ATCACCGAAA CGCGCGAGGC AGCAAGGAGA TGGCGCCCAA 6819
CAGTCCCCCG GCCACGGGGC CTGCCACCAT ACCCACGCCG AAACAAGCGC TCATGAGCCC 6879
GAAGTGGCGA GCCCGATCTT CCCCATCGGT GATGTCGGCG ATATAGGCGC CAGCAACCGC 6939
ACCTGTGGCG CCGGTGATGC CGGCCACGAT GCGTCCGGCG TAGAGGATCT GCTCATGTTT 6999
GACAGCTTAT C 7010

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 227 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

```
Met Thr Pro Asp Ile Ile Leu Gln Arg Thr Gly Ile Asp Val Arg Ala
1               5                   10                  15

Val Glu Gln Gly Asp Asp Ala Trp His Lys Leu Arg Leu Gly Val Ile
            20                  25                  30

Thr Ala Ser Glu Val His Asn Val Ile Ala Lys Pro Arg Ser Gly Lys
        35                  40                  45

Lys Trp Pro Asp Met Lys Met Ser Tyr Phe His Thr Leu Leu Ala Glu
    50                  55                  60

Val Cys Thr Gly Val Ala Pro Glu Val Asn Ala Lys Ala Leu Ala Trp
65                  70                  75                  80

Gly Lys Gln Tyr Glu Asn Asp Ala Arg Thr Leu Phe Glu Phe Thr Ser
                85                  90                  95

Gly Val Asn Val Thr Glu Ser Pro Ile Ile Tyr Arg Asp Glu Ser Met
            100                 105                 110

Arg Thr Ala Cys Ser Pro Asp Gly Leu Cys Ser Asp Gly Asn Gly Leu
        115                 120                 125
```

```
Glu Leu Lys Cys Pro Phe Thr Ser Arg Asp Phe Met Lys Phe Arg Leu
    130                 135                 140

Gly Gly Phe Glu Ala Ile Lys Ser Ala Tyr Met Ala Gln Val Gln Tyr
145                 150                 155                     160

Ser Met Trp Val Thr Arg Lys Asn Ala Trp Tyr Phe Ala Asn Tyr Asp
                165                 170                 175

Pro Arg Met Lys Arg Glu Gly Leu His Tyr Val Val Ile Glu Arg Asp
            180                 185                 190

Glu Lys Tyr Met Ala Ser Phe Asp Glu Ile Val Pro Glu Phe Ile Glu
        195                 200                 205

Lys Met Asp Glu Ala Leu Ala Glu Ile Gly Phe Val Phe Gly Glu Gln
    210                 215                 220

Trp Arg  *
225
```

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 262 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

```
Met Ser Thr Ala Leu Ala Thr Leu Ala Gly Lys Leu Ala Glu Arg Val
  1               5                  10                  15

Gly Met Asp Ser Val Asp Pro Gln Glu Leu Ile Thr Thr Leu Arg Gln
            20                  25                  30

Thr Ala Phe Lys Gly Asp Ala Ser Asp Ala Gln Phe Ile Ala Leu Leu
        35                  40                  45

Ile Val Ala Asn Gln Tyr Gly Leu Asn Pro Trp Thr Lys Glu Ile Tyr
    50                  55                  60

Ala Phe Pro Asp Lys Gln Asn Gly Ile Val Pro Val Val Gly Val Asp
65                  70                  75                  80

Gly Trp Ser Arg Ile Ile Asn Glu Asn Gln Gln Phe Asp Gly Met Asp
                85                  90                  95

Phe Glu Gln Asp Asn Glu Ser Cys Thr Cys Arg Ile Tyr Arg Lys Asp
            100                 105                 110

Arg Asn His Pro Ile Cys Val Thr Glu Trp Met Asp Glu Cys Arg Arg
        115                 120                 125

Glu Pro Phe Lys Thr Arg Glu Gly Arg Glu Ile Thr Gly Pro Trp Gln
    130                 135                 140

Ser His Pro Lys Arg Met Leu Arg His Lys Ala Met Ile Gln Cys Ala
145                 150                 155                 160

Arg Leu Ala Phe Gly Phe Ala Gly Ile Tyr Asp Lys Asp Glu Ala Glu
                165                 170                 175
```

Arg Ile Val Glu Asn Thr Ala Tyr Thr Ala Glu Arg Gln Pro Glu Arg
180 185 190

Asp Ile Thr Pro Val Asn Asp Glu Thr Met Gln Glu Ile Asn Thr Leu
195 200 205

Leu Ile Ala Leu Asp Lys Thr Trp Asp Asp Asp Leu Leu Pro Leu Cys
210 215 220

Ser Gln Ile Phe Arg Arg Asp Ile Arg Ala Ser Ser Glu Leu Thr Gln
225 230 235 240

Ala Glu Ala Val Lys Ala Leu Gly Phe Leu Lys Gln Lys Ala Ala Glu
245 250 255

Gln Lys Val Ala Ala *
260

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 139 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Met Asp Ile Asn Thr Glu Thr Glu Ile Lys Gln Lys His Ser Leu Thr
1 5 10 15

Pro Phe Pro Val Phe Leu Ile Ser Pro Ala Phe Arg Gly Arg Tyr Phe
20 25 30

His Ser Tyr Phe Arg Ser Ser Ala Met Asn Ala Tyr Tyr Ile Gln Asp
35 40 45

Arg Leu Glu Ala Gln Ser Trp Ala Arg His Tyr Gln Gln Leu Ala Arg
50 55 60

Glu Glu Lys Glu Ala Glu Leu Ala Asp Asp Met Glu Lys Gly Leu Pro
65 70 75 80

Gln His Leu Phe Glu Ser Leu Cys Ile Asp His Leu Gln Arg His Gly
85 90 95

Ala Ser Lys Lys Ser Ile Thr Arg Ala Phe Asp Asp Asp Val Glu Phe
100 105 110

Gln Glu Arg Met Ala Glu His Ile Arg Tyr Met Val Glu Thr Ile Ala
115 120 125

His His Gln Val Asp Ile Asp Ser Glu Val *
130 135

Table 1: Sequences of Oligos for PCR

Figure 3ab
left: TGACCCCTCACAAGGAGACGACCTTCCATGACCGAGTACAAGAGGGATGTAACGCACTGA
right: TACAAATGTGGTATGGCTGATTATGATCCTCTAGAGTCGGTGCTCACTGCCCGCTTTCCA
template: pJP5603
targeting vector: pSV-paz11

Figure 3c
a-left: CTTCCATGACCGAGTACAAGAGGGATGTAACGCACTGA
a-right: ATGATCCTCTAGAGTCGGTGCTCACTGCCCGCTTTCCA
b-left: AGACGACCTTCCATGACCGAGTACAAGAGGGATGTAACGCACTGA
b-right: GCTGATTATGATCCTCTAGAGTCGGTGCTCACTGCCCGCTTTCCA
c-left: CACAAGGAGACGACCTTCCATGACCGAGTACAAGAGGGATGTAACGCACTGA
c-right: TGGTATGGCTGATTATGATCCTCTAGAGTCGGTGCTCACTGCCCGCTTTCCA
d-left: TGACCCCTCACAAGGAGACGACCTTCCATGACCGAGTACAAGAGGGATGTAACGCACTGA
d-right: TACAAATGTGGTATGGCTGATTATGATCCTCTAGAGTCGGTGCTCACTGCCCGCTTTCCA
e-left:
CACGCCCCTGACCCCTCACAAGGAGACGACCTTCCATGACCGAGTACAAGAGGGATGTAACGCACTGA
e-right:
TAAAACCTCTACAAATGTGGTATGGCTGATTATGATCCTCTAGAGTCGGTGCTCACTGCCCGCTTTCCA
f-left:
TCCCCTGACCCACGCCCCTGACCCCTCACAAGGAGACGACCTTCCATGACCGAGTACAAGAGGGATGT
AACGCACTGA
f-right:
TAAAGCAAGTAAAACCTCTACAAATGTGGTATGGCTGATTATGATCCTCTAGAGTCGGTGCTCACTGCC
CGCTTTCCA
template: pJP5603
targeting vector: pSV-paz11

Figure 3d
a-left:
TCATCCTCTGCATGGTCAGGTCATGGATGAGCAGACGATGGTGCAGGATCAAGGGCTGCTAAAGGAA
a-right:
TAATGCGAACAGCGCACGGCGTTAAAGTTGTTCTGCTTCATCAGCAGGATGGCGAAGAACTCCAGCAT
b-left:
CACGAGCATCATCCTCTGCATGGTCAGGTCATGGATGAGCAGACGATGGCAAGGGCTGCTAAAGGAA
b-right:
TAATGCGAACAGCGCACGGCGTTAAAGTTGTTCTGCTTCATCAGCAGGATGGCGAAGAACTCCAGCAT
c-left:
TTAACCGTCACGAGCATCATCCTCTGCATGGTCAGGTCATGGATGAGCACAAGGGCTGCTAAAGGAA
c-right:
TAATGCGAACAGCGCACGGCGTTAAAGTTGTTCTGCTTCATCAGCAGGATGGCGAAGAACTCCAGCAT
d-left:
TGCTGCTGAACGGCAAGCCGTTGCTGATTCGAGGCGTTAACCGTCACGACAAGGGCTGCTAAAGGAA
d-right:
TAATGCGAACAGCGCACGGCGTTAAAGTTGTTCTGCTTCATCAGCAGGATGGCGAAGAACTCCAGCAT
e-left:
TCTCTATCGTGCGGTGGTTGAACTGCACACCGCCGACGGCACGCTGATTCAAGGGCTGCTAAAGGAA
e-right:
TAATGCGAACAGCGCACGGCGTTAAAGTTGTTCTGCTTCATCAGCAGGATGGCGAAGAACTCCAGCAT
f-left:
TGGAGTGACGGCAGTTATCTGGAAGATCAGGATATGTGGCGGATGAGCGCAAGGGCTGCTAAAGGAA

f-right:
TAATGCGAACAGCGCACGGCGTTAAAGTTGTTCTGCTTCATCAGCAGGATGGCGAAGAACTCCAGCAT
g-left:
TGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCAAGGGCTGCTAAAGGAA
g-right:
TAATGCGAACAGCGCACGGCGTTAAAGTTGTTCTGCTTCATCAGCAGGATGGCGAAGAACTCCAGCAT
h-left:
TGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCAAGGGCTGCTAAAGGAA
h-right:
TATTTTTGACACCAGACCAACTGGTAATGGTAGCGACCGGCGCTCAGCTGGCGAAGAACTCCAGCAT
template: pJP5603
targeting vector: pSV-paz11

Figure 4
left:
TCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAGCTTATGCCCACCAGC
TGGTATGGCTGATTATGATC
right:
TCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCGGGCAATCAGGTGCGACA
ATCTACCACCAGCTCTTTTCTACGGGGTCTGACGC
template: pBR322
targeting vector: Hoxa-P1

Figure 5
left:
TGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTTAATACGACTCACTATAGGGAGAACA
GGAAACAGCTATGCCCATAACACCCAGAGTA
right:
TGCGCCGCTACAGGGCGCGTCCATTCGCCATTCAGGCCTGACTCACTAGTGATGGTGATGGTGATGTGG
GGGGTGCCGCTCAGT
template: pmtrx (a pBluescipt vector carrying mouse trithorax cDNA)
targeting vector: pZero2.1

Figure 6
left:
TGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGGAGAAAAAAATCACT
GGATATACCACCG
right:
TACAGGGCGCGTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACGCCCCGCCCTGC
CACTCATCGCA
template: pMAK705
targeting vector: pBAD-24 backbone Amp resistant gene

Figure 8
i:
TGCCAAGCTTGACCCACTGTGGAAGTGTTCCAAAAAGCGGGAAGGCTCTTGAGCTACTTCACTAACAAC
CGG
g:
TCACCATCTTCGGGCCATTTGTAGACTGGAATATTTCGAGCTATGAGTGTGCTACTTCACTAACAACCG
G
h:
TGGCCCCAGGGTGACGCGGACATGGAGTTGTCGGCCAGGGCACTGGTCCATGAGAGTGCCAAGCTACTC
GCGAC
template: pKaZ
targeting vector: Hoxa-P1

Figure 9

j:

TAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCT
TTGCCTGGTTTATAACTTCGTATAGCATACATTATACGAAGTTATGGGCTGCTAAAGGAAGCGGAACAC
G

k:

TGGCAGTTCAGGCCAATCCGCGCCGGATGCGGTGTATCGCTCGCCACTTCAACATCAACGGTAATCGCC
ATTTGACCATATAACTTCGTATAATGTATGCTATACGAAGTTATCCCCAGAGTCCCGCTCAGAAGAACT

template: pJP5603

targeting vector: JC9604 chromosome

Figure 10

l:

TAGCTTGGCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACCCATCAC
ATATACCTGCCGTTCACTAT

m:

TATCGGTGGCCGTGGTGTCGGCTCCGCCGCCTTCATACTGCACCGGGCGGGAAGGCGATTCCGAAGCCC
AACCTTTCATAGAAGCC

template: pIB279

targeting vector: pSV-paX1

l*: GCTTGGCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAA

m*:

TCGGTGGCCGTGGTGTCGGCTCCGCCGCCTTCATACTGCACCGGGCGGGAAGGATCCACAGATTTGATC
CAGCGATACAGC

template: pSV-paz11

targeting vector: pSV-sacB-neo

Figure11

n:

TACCGCATTAAAGCTTATCGATGATAAGCTGTCAAACATGAGAATTGACCCGGAACCCTTCTCGAGGAA
GTTCCTATTCTCTAGAAAGTATAGGAACTTCCGAATAAATACCTGTGACGGAAGATCACTT

p:

TTCCCTCAAGAATTTTACTCTGTCAGAAACGGCCTTAACGACGTAGTCGAGGGACCTAGAAGTTCCTAT
ACTTTCTAGAGAATAGGAACTTCATTATCACTTATTCAGGCGTAGCACCAGGCG

template: pMAK705

targeting vector: Hoxa-P1

Figure 12

left:

TGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGGAGAAAAAAATCACT
GGATATACCACCG

right:

TACAGGGCGCGTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACGCCCCGCCCTGC
CACTCATCGCA

template: pMAK705

targeting vector: pBAD-24 backbone Amp resistant gene

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(71) Applicant (for all designated States except US): EUROPÄISCHES LABORATORIUM FÜR MOLEKULARBIOLOGIE (EMBL) [DE/DE]; Meyerhofstrasse 1, D-69117 Heidelberg (DE).

(72) Inventors; and
(75) Inventors/Applicants (for US only): STEWART, Francis [AU/DE]; Lärchenweg 3, D-69181 Leimen (DE). ZHANG, Youming [CN/DE]; Friedrich-Ebert-Anlage 51e, D-69117 Heidelberg (DE). BUCHHOLZ, Frank [DE/DE]; Neuenkirchener Weg 44a, D-28779 Bremen (DE).

(74) Agents: WEICKMANN, H. et al.; Kopernikusstrasse 9, D-81679 München (DE).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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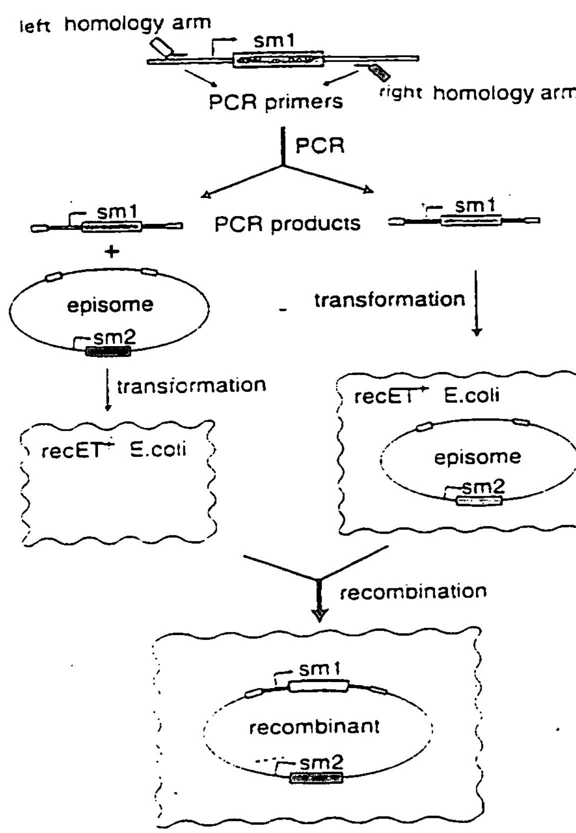
(57) Abstract

The invention refers to a novel method for cloning DNA molecules using a homologous recombination mechanism between at least two DNA molecules comprising: a) providing a host cell capable of performing homologous recombination, b) contacting in said host cell a first DNA molecule which is capable of being replicated in said host cell with a second DNA molecule comprising at least two regions of sequence homology to regions on the first DNA molecule, under conditions which favour homologous recombination between said first and second DNA molecules and c) selecting a host cell in which homologous recombination between said first and second DNA molecules has occurred. In particular, it relies on the use of the E. coli RecE and RecT proteins, the bacteriophage Red-alpha and Red-beta proteins, or the phage P22 recombination system. The beneficial effects of concomitant expression of the RecBC inhibitor genes (e.g. Red-Gamma) is also examplified.



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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 98/07945

A. CLASSIFICATION OF SUBJECT MATTER
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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	OLINER J.D. ET AL.: "In vivo Cloning of PCR Products in E. coli" NUCLEIC ACIDS RESEARCH, vol. 21, no. 22, 1993, pages 5192-5197, XP002064297 cited in the application	1-6, 8-13, 19, 20, 22-27, 34, 35, 37-42, 46-48, 50
Y	See page 5192, column 2, line 8 - line 31, and discussion section	18, 28

Y	NUSSBAUM A ET AL: "Restriction-stimulated homologous recombination of plasmids by the RecE pathway of Escherichia coli" GENETICS, vol. 130, no. 1, January 1992, pages 37-49, XP002103370 see figures 1,7	18, 28

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[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
21 May 1999	04/06/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lonnoy, O

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International Application No
PCT/EP 98/07945

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	ZHANG Y ET AL: "A new logic for DNA engineering using recombination in Escherichia coli" NATURE GENETICS, vol. 20, no. 2, October 1998, pages 123-128, XP002103371 see the whole document	1-50
A	KOLODNER R ET AL: "Homologous pairing proteins encoded by the Escherichia coli recE and recT genes" MOLECULAR MICROBIOLOGY, vol. 11, no. 1, 1994, pages 23-30, XP002064301 cited in the application	
A	LUISI-DELUCA C ET AL: "Genetic and physical analysis of plasmid recombination in recB recC sbcB and recB recC sbcA Escherichia coli K-12 mutants" GENETICS, vol. 122, 1989, pages 269-278, XP002064305	
A	DEGRYSE E: "Evaluation of Escherichia coli recBC sbcBC mutants for cloning by recombination in vivo" JOURNAL OF BIOTECHNOLOGY, vol. 2, no. 39, 15 April 1995, page 181-187 XP004036984	
A	YANG X ET AL: "Homologous recombination based modification in Escherichia coli and germ line transmission in transgenic mice of a bacterial artificial chromosome" NATURE BIOTECHNOLOGY, vol. 15, September 1997, pages 859-865, XP002103372	
A	MURPHY K: "Lambda Gam protein inhibits the helicase and Chi-stimulated recombination activities of Escherichia coli RecBCD enzyme" JOURNAL OF BACTERIOLOGY, vol. 173, no. 18, September 1991, pages 5808-5821, XP002103373	

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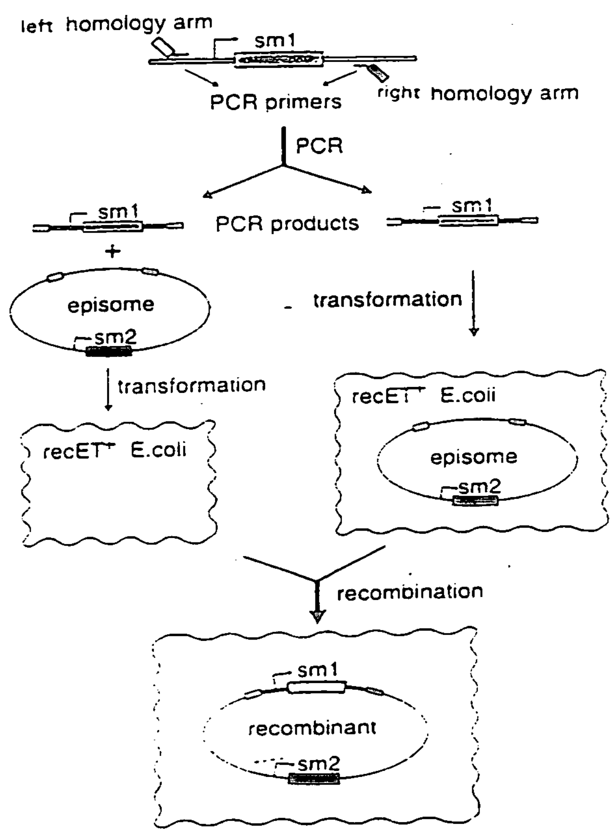

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EE	Estonia						

AMENDED CLAIMS

[received by the International Bureau on 04 August 1999 (04.08.99); original claims 1-50 replaced by new claims 1-64 (10 pages)]

1. A method for cloning DNA molecules in procaryotic cells comprising the steps of:
   a) providing a procaryotic host cell capable of performing homologous recombination,
   b) contacting in said host cell a circular first DNA molecule which is capable of being replicated in said host cell with a second DNA molecule comprising at least two regions of sequence homology to regions on the first DNA molecule, under conditions which favour homologous recombination between said first and second DNA molecules and
   c) selecting a host cell in which homologous recombination between said first and second DNA molecules has occurred.

2. The method according to claim 1 wherein the homologous recombination occurs via the recET cloning mechanism.

3. The method according to claim 2 wherein the host cell is capable of expressing recE and recT genes.

4. The method according to claim 3 wherein the recE and recT genes are selected from E.coli recE and recT genes or from λ redα and redß genes.

5. The method according to claim 3 or 4 wherein the host cell is transformed with at least one vector capable of expressing recE and/or recT genes.

6. The method of claim 3, 4 or 5 wherein the expression of the recE and/or recT genes is under control of a regulatable promoter.

7. The method of claim 5 or 6 wherein the recT gene is overexpressed versus the recE gene.

8. The method according to any one of claims 3 to 7 wherein the recE gene is selected from a nucleic acid molecule comprising
(a) the nucleic acid sequence from position 1320 (ATG) to 2159 (GAC) as depicted in Fig.7B,
(b) the nucleic acid sequence from position 1320 (ATG) to 1998 (CGA) as depicted in Fig.13B,
(c) a nucleic acid encoding the same polypeptide within the degeneracy of the genetic code and/or
(d) a nucleic acid sequence which hybridizes under stringent conditions with the nucleic acid sequence from (a), (b) and/or (c).

9. The method according to any one of claims 3 to 8 wherein the recT gene is selected from a nucleic acid molecule comprising
(a) the nucleic acid sequence from position 2155 (ATG) to 2961 (GAA) as depicted in Fig.7B,
(b) the nucleic acid sequence from position 2086 (ATG) to 2868 (GCA) as depicted in Fig.13B,
(c) a nucleic acid encoding the same polypeptide within the degeneracy of the genetic code and/or
(d) a nucleic acid sequence which hybridizes under stringent conditions with the nucleic acid sequences from (a), (b) and/or (c).

10. The method according to any one of the previous claims wherein the host cell is a gram-negative bacterial cell.

11. The method according to claim 10 wherein the host cell is an Escherichia coli cell.

12. The method according to claim 11 wherein the host cell is an Escherichia coli K12 strain.

13. The method according to claim 12 wherein the E.coli strain is selected from JC 8679 and JC 9604.

14. The method according to any one of the previous claims wherein the host cell further is capable of expressing a recBC inhibitor gene.

15. The method according to claim 14 wherein the host cell is transformed with a vector expressing the recBC inhibitor gene.

16. The method according to claim 14 or 15 wherein the recBC inhibitor gene is selected from a nucleic acid molecule comprising
(a) the nucleic acid sequence from position 3588 (ATG) to 4002 (GTA) as depicted in Fig.13B,
(b) a nucleic acid encoding the same polypeptide within the degeneracy of the genetic code and/or
(c) a nucleic acid sequence which hybridizes under stringent conditions (as defined above) with the nucleic acid sequence from (a) and/ or (b).

17. The method according to any one of claims 13 to 16 wherein the host cell is a prokaryotic recBC+ cell.

18. The method according to any one of the previous claims wherein the first DNA molecule is an extrachromosomal DNA molecule containing an origin of replication which is operative in the host cell.

19. The method according to claim 18 wherein the first DNA molecule is selected from plasmids, cosmids, P1 vectors, BAC vectors and PAC vectors.

20. The method according to any one of claims 1-18 wherein the first DNA molecule is a host cell chromosome.

21. The method according to any one of the previous claims wherein the second DNA molecule is linear.

22. The method according to any one of the previous claims wherein the regions of sequence homology are at least 15 nucleotides each.

23. The method according to one of claims 1 to 16 wherein the second DNA molecule is obtained by an amplification reaction.

24. The method according to one of the previous claims wherein the first and/or second DNA molecules are introduced into the host cells by transformation.

25. The method according to claim 24 wherein the transformation method is electroporation.

26. The method according to one of claims 1 to 25 wherein the first and second DNA molecules are introduced into the host cell simultaneously by co-transformation.

27. The method according to one of claims 1 to 25 wherein the second DNA molecule is introduced into a host cell in which the first DNA molecule is already present.

28. The method according to one of the previous claims wherein the second DNA molecule contains at least one marker gene placed between the two regions of s :quence homology and wherein homologous recombination is detected by expression of said marker gene.

29. The method according to claim 28 wherein gene presence is selected from antibiotic resistance genes, deficiency complementation genes and reporter genes.

30. The method of any one of claims 1 to 29 wherein the first DNA molecule contains at least one marker gene between the two regions of sequence homology and wherein homologous recombination is detected by lack of expression of said marker gene.

31. The method of any one of claims 1 to 30 wherein said marker gene is selected from genes which, under selected conditions, convey a toxic or bacteriostatic effect on the cell, and reporter genes.

32. A method according to any one of the previous claims wherein the first DNA molecule contains at least one target site for a site specific recombinase between the two regions of sequence homology and wherein homologous recombination is detected by removal of said target site.

33. A method for cloning DNA molecules comprising the steps of:
(a) providing a source of RecE and RecT proteins,
(b) contacting a first DNA molecule which is capable of being replicated in a suitable host cell with a second DNA molecule comprising at least two regions of sequence homology to regions on the first DNA molecule, under conditions which favour homologous recombination between said first and second DMA molecules and
(c) selecting DNA molecules in which homologous recombination between said first and second DNA molecules has occurred.

34. The method of claim 33 wherein said RecE and RecT or proteins are selected from E.coli RecE and RecT proteins or from phage λ Redα and Redß proteins.

35. The method of claim 33 or 34 wherein the recombination occurs in vitro.

36. The method of claim 33 or 34 wherein the recombination occurs in vivo.

37. A method for making a recombinant DNA molecule comprising introducing into a prokaryotic host cell a circular first DNA molecule which is capable of being replicated in said host cell, and introducing a second DNA molecule comprising a first and a second region of sequence homology to a third and fourth region, respectively, on the first DNA molecule, said host cell being capable of performing homologous recombination, such that a recombinant DNA molecule is made, said recombinant DNA molecule comprising the first DNA molecule wherein the sequences between said third and fourth regions have been replaced by sequences between the first and second regions of the second DNA molecule.

38. The method according to claim 37 which further comprises detecting the recombinant DNA molecule.

39. A method for making a recombinant DNA molecule comprising introducing into a prokaryotic host cell, containing a chromosomal first DNA molecule, a second DNA molecule comprising a first and a second region of sequence homology to a third and a fourth region, respectively, on the host chromosomal first DNA molecule, said host cell being capable of performing homologous recombination, such that a recombinant DNA molecule is made, said recombinant DNA molecule comprising the chromosomal first DNA molecule wherein the sequences between said third and fourth regions have been replaced by sequences between the first and second regions of the second DNA molecule.

40. The method according to claim 39 which further comprises detecting the recombinant DNA molecule.

41. The method according to any one of claims 37 to 40, wherein the host cell is capable of expressing RecE and RecT proteins or λexo and λß proteins.

42. A method for cloning DNA molecules comprising the steps of:
   (a) contacting in vitro a first DNA molecule with a second DNA molecule comprising at least two regions of sequence homology to regions on the first DNA molecule, in the presence of RecE and RecT proteins and under conditions which favour homologous recombination between said first and second DNA molecules; and
   (b) selecting a DNA molecule in which homologous recombination between said first and second DNA molecules has occurred.

43. A method for making a recombinant DNA molecule comprising contacting in vitro a first DNA molecule with a second DNA molecule comprising a first and a second region of sequence homology to a third and a fourth region on the first DNA molecule, in the presence of RecE and RecT proteins and under conditions in which homologous recombination can occur, such that a recombinant DNA molecule is made, said recombinant DNA molecule comprising the first DNA molecule wherein the sequences between said third and fourth regions have been replaced by sequences between the first and second regions of the second DNA molecule.

44. The method of claim 42, which further comprises between steps (a) and (b) the step of introducing the product step (a) into a cell, wherein recombination occurs in the cell.